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Immobilized enzyme In PDMS substrate reaction modeling and Simulation for specific protein detection in biosensor

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Dedication

To my beloved parents

Who wait so long to see successful project carried their son

To my family and friends

Who stand with me in good and bad

To my teacher's

For their support and encouragement

Ghalem Mahfoud

Acknowledgement

My prays and Thanks should be first and last to ALLAH, the almighty most gracious and most merciful who enabled me to conduct the project by the grace of him, giving me the confidence and patience to accomplish this work.

All gratitude and respect to my supervisor and co_supervisor Dr. A. Hassein-Bey & Asma. L. S. Hassein-Bey respectively, for their supervision and constant support to help me to bring up this work and all teachers and staff of Physics department . I would also to thank all the jury member and also Dr.Hakim Tahi for being here with us today.

My sincere thanks go to my family and friends for their continuous support.

I'm indebted to my wonderful mother and father for their patience, encouragement and moral support during this project.

Abstract

Many enzyme catalyzed reactions are exothermic, generating very small amounts of heat. Biosensors based on the measurement of this heat can arrive at the detection and measurement of analyte concentration. This represents the type of biosensor most generally applicable.

The work of this project will consist in implementing the various multiphysical phenomena (fluid mechanics, microfluidics, heat exchange, enzyme-substrate exothermic reaction, electro-resistive transduction of sensitive layers, ...) in comsol multiphysics. Starting from previous works on the study and the simulation of the dilution of species in microchannel, try to arrive to predict by simulation by the method of the finite elements the behavior of the thermosensitive layer based on materials with phase transition.

ملخص

العديد من تفاعلات الإنزيم المحفز طاردة للحرارة بكميات قليلة جدًا . يمكن أن تصل أجهزة الاستشعار البيولوجي استنادًا إلى قياس هذه الحرارة إلى كشف وقياس تركيز الحليلة. هذا يمثل نوع المستشعر الحيوي الأكثر قابلية للتطبيق بشكل عام.

الهدف من هذا العمل هو تنفيذ مختلف الظواهر الفيزيائية (ميكانيكا السوائل، وعلى الموائع الجزيئية ، تبادل الحرارة، والطاردة للحرارة رد فعل الانزيم الركيزة، التحويل الكهربائي المقاوم للطبقات الحساسة ، ...) في COMSOL. بدءا من الأعمال السابقة القائمة على دراسة ومحاكاة تمييع الأنواع المتناهية، في محاولة للوصول الى تقديم محاكاة سلوك الطبقة الحساسة للحرارة على أساس المواد باستخدام طريقة العناصر المحدودة.

Résumé

De nombreuses réactions catalysées par des enzymes sont exothermiques, générant de très petites quantités de chaleur. Des biocapteurs basés sur la mesure de cette chaleur peuvent arriver à la détection et mesure de concentration d'analyte. Ceci représente le type de biocapteur le plus généralement applicable. Le travail de ce projet consistera à implémenter les différents phénomènes multiphysiques (mécanique des fluides, microfluidique, échange de chaleurs, réaction exothermique enzyme-substrat, transduction électro-résistive des couches sensibles, ...) dans comsol multiphysics. En partant de travaux précédents sur l'étude et la simulation de la dilution d'espèces dans microcanal, essayer d'arriver à prévoir par simulation par la méthode des éléments finis le comportement de la couche thermosensible à base de matériaux à transition de phase.

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CHAPTER 1

STATE OF ART OF BIOSENSOR

1.1 Introduction

A biosensor is a device that measures biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction. Biosensors are employed in applications such as disease monitoring, drug discovery, and detection of pollutants, disease-causing micro-organisms and markers that are indicators of a disease in bodily fluids (blood, urine, saliva, sweat). A typical biosensor is represented in Figure 1.1; it consists of the following components.

- **Analyte**: A substance of interest that needs detection. For instance, glucose is an ‘analyte’ in a biosensor designed to detect glucose.
- **Bioreceptor**: A molecule that specifically recognises the analyte is known as a bioreceptor. Enzymes, cells, aptamers, deoxyribonucleic acid (DNA) and antibodies are some examples of bioreceptors. The process of signal generation (in the form of light, heat, pH, charge or mass change, etc.) upon interaction of the bioreceptor with the analyte is termed bio-recognition.
- **Transducer**: The transducer is an element that converts one form of energy into another. In a biosensor the role of the transducer is to convert the bio-recognition event into a measurable signal. This process of energy conversion is known as signalisation. Most transducers produce either optical or electrical signals that are usually proportional to the amount of analyte–bioreceptor interactions.
- **Electronics**: This is the part of a biosensor that processes the transduced signal and prepares it for display. It consists of complex electronic circuitry that performs signal conditioning such as amplification and conversion of signals from analogue into the digital form. The processed signals are then quantified by the display unit of the biosensor.
- **Display**: The display consists of a user interpretation system such as the liquid crystal display of a computer or a direct printer that generates numbers or curves understandable by the user. This part often consists of a combination of hardware and software that generates results of the biosensor in a user-friendly manner. The output signal on the display can be numeric, graphic, tabular or an image, depending on the requirements of the end user [1] .

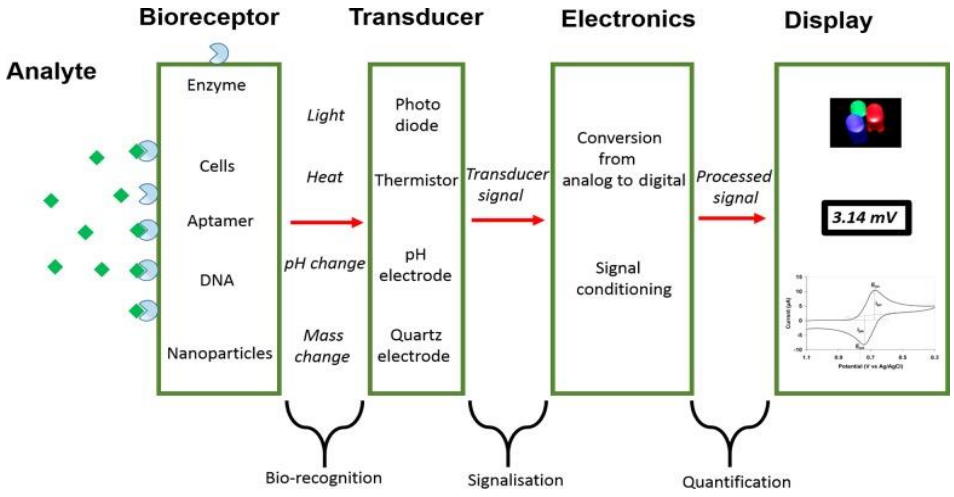

Figure 1.1: Schematic representation of a biosensor[1].

1.2 Historical Background

The history of biosensors dates back to as early as 1906 when M. Cremer [2] demonstrated that the concentration of an acid in a liquid is proportional to the electric potential that arises between parts of the fluid located on opposite sides of a glass membrane. However, it was only in 1909 that the concept of pH (hydrogen ion concentration) was introduced by Søren Peder Lauritz Sørensen and an electrode for pH measurements was realised in the year 1922 by W.S. Hughes [3]. Between 1909 and 1922, Griffin and Nelson [4,5] first demonstrated immobilisation of the enzyme invertase on aluminium hydroxide and charcoal. The first 'true' biosensor was developed by Leland C. Clark, Jr in 1956 for oxygen detection. He is known as the 'father of biosensors' and his invention of the oxygen electrode bears his name: 'Clark electrode' [6]. The demonstration of an amperometric enzyme electrode for the detection of glucose by Leland Clark in 1962 was followed by the discovery of the first potentiometric biosensor to detect urea in 1969 by Guilbault and Montalvo, Jr [7]. Eventually in 1975 the first commercial biosensor was developed by Yellow Spring Instruments (YSI). **Table 1.1** shows the historical overview of biosensors in the period 1970–1992. Ever since the development of the i-STAT sensor, remarkable progress has been achieved in the field of biosensors. The field is now a multidisciplinary area of research that bridges the principles of basic sciences (physics, chemistry and biology) with fundamentals of micro/nano-technology, electronics and applicatory medicine. The database 'Web of Science' has indexed over 84000 reports on the topic of 'biosensors' from 2005 to 2015.

1970	Discovery of ion-sensitive field-effect transistor (ISFET) by Bergveld [8]
1975	Fibre-optic biosensor for carbon dioxide and oxygen detection by Lubbers and Opitz [9]

1975	First commercial biosensor for glucose detection by YSI [10]
1975	First microbe-based immunosensor by Suzuki et al. [11]
1982	Fibre-optic biosensor for glucose detection by Schultz [12]
1983	Surface plasmon resonance (SPR) immunosensor by Liedberg et al. [13]
1984	First mediated amperometric biosensor: ferrocene used with glucose oxidase for glucose detection [14]
1990	SPR-based biosensor by Pharmacia Biacore [9]
1992	Handheld blood biosensor by i-STAT [9]

Table 1.1: the historical overview of biosensors in the period 1970–1992 .

1.3 Biosensor characteristics

Biosensors are usually characterized by the following parameters:

• **Sensitivity:** is the response of the sensor to changes in analyte concentration.

• **Selectivity:** is the ability of the sensor to respond only to the target analyte. That is, lack of response to other interfering chemicals is the desired feature.

• **Range:** is the concentration range over which the sensitivity of the sensor is good. Sometimes this is called dynamic range or linearity.

• **Response time:** is the time required for the sensor to indicate 63% of its final response due to a step change in analyte concentration.

• **Reproducibility:** is the accuracy with which the sensor's output can be obtained.

• **Detection limit:** is the lowest concentration of the analyte to which there is a measurable response.

• **Life time:** is the time period over which the sensor can be used without significant deterioration in performance characteristics.

Stability: characterizes the change in its baseline or sensitivity over a fixed period of time [15].

1.4 Type of Biosensors

Biosensors can be broadly categorized as either transducer or bioreceptor which can bioaffinity devices or biocatalytic devices as shown in **Figure (1.2)**. In the bioaffinity devices, the analyte in the solution binds selectively to a receptor immobilized on the biosensor surface. In the biocatalytic devices, an enzyme immobilized on the biosensor surface catalysis the target substance [15] .

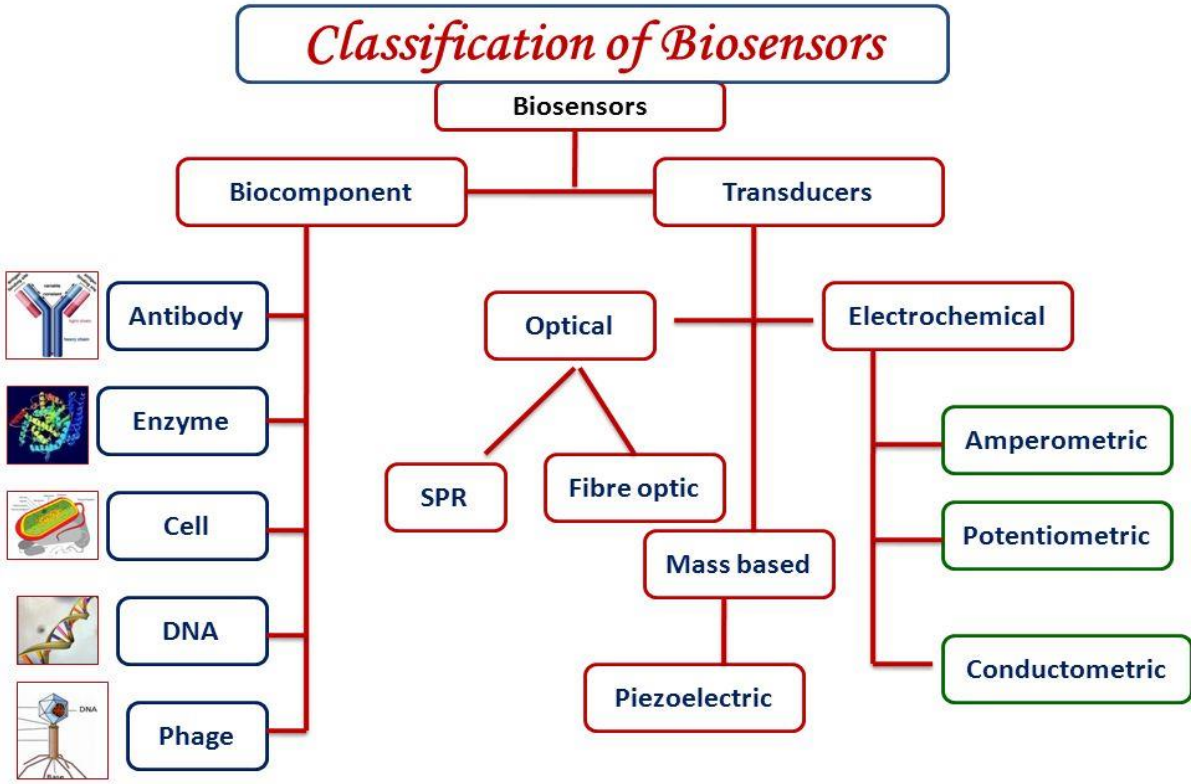

Figure1.2: Classification of biosensor [16] .

1.5 Biosensors According to the transducer type

1.5.1 Electrochemical biosensors

Electrochemical is very big field and there a lot of applications, currently applications electrochemical sensor by using COMSOL one of those used is applications Amperometric with potentiometric transducers are the largely usually used electrochemical transducers. as well In amperometric transducers, can the possible between the two electrodes is set and the current produced by the rust or decrease of electro active type is measured and can related to the concentration of the study of attention. In other hand, platinum, silver, gold, or carbon, be able to are create electrodes of -based materials that are inert at the potentials at which the electrochemical reply takes place. Is very low current of the Potentiometric transducers measure the potential of electrochemical cells Field effect transistors (FET) are potentiometric devices based on the measurement of potential at an insulator–electrolyte interface. Also the metal gate of a FET can be substituted by an ion selective membrane to make a pH transducer (pH ISFET). They can show Enzymes on the face of such pH ISFET to create enzyme sensitized field effect transistors (ENFET). [17] The figure below shown Basic principle of electrochemical biosensor.

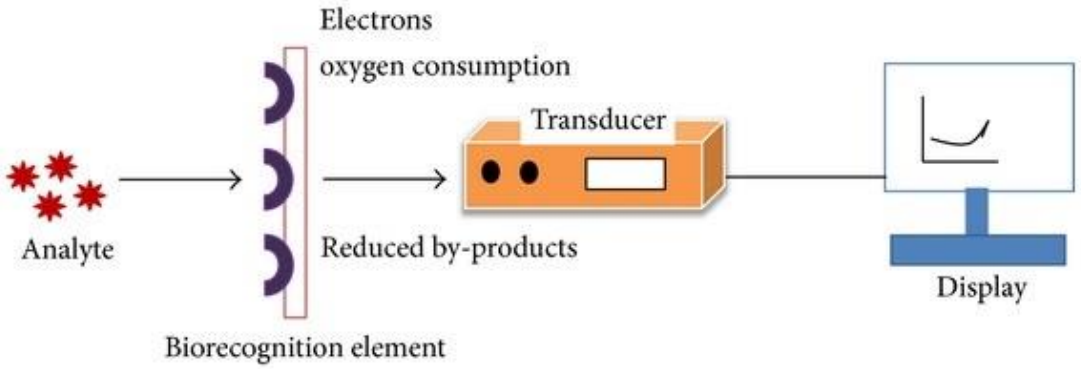

Figure 1.3: Basic principle of electrochemical biosensor[18] .

1.5.2 Amperometric Biosensors

In amperometry, the current produced by the oxidation or reduction of an electro active analyte species at an electrode surface is monitored under controlled potential conditions. The magnitude of the current is then related to the quantity of analyte present [19]. Clark oxygen electrodes perhaps represent the basis for the simplest forms of amperometry biosensors, where a current is produced in proportion to the oxygen concentration. This is measured by the reduction of oxygenate a platinum working electrode in reference to a Ag/AgCl reference electrode at a given potential. Typically, the current is measured at a constant potential and this is referred to as amperometry. If a current is measured during controlled variations of the potential, this is referred to as voltammetry.

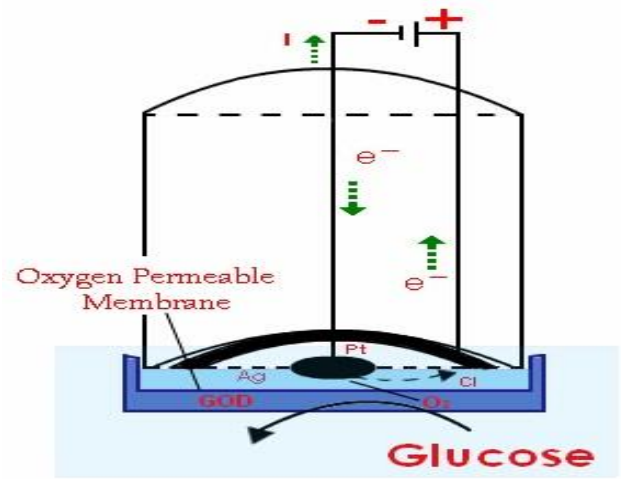

Figure 1.4: Example of an amperometric biosensor [20] .

1.5.3 Potentiometric Biosensors

These biosensors are based on ion-selective electrodes (ISE) and ion-sensitive field effect transistors (ISFET). The primary outputting signal is possibly due to ions accumulated at the ion-selective membrane interface. Current flowing through the electrode is equal to or near zero. The electrode follows the presence of the monitored ion resulting from the enzyme reaction. For example, glucose oxidize can be immobilized on a surface of the pH electrode. Glucose has only minimal influence on pH in the working medium; however, the enzymatically formed gluconate causes acidification. A biorecognition element is immobilized on the outer surface or captured inside the membrane.

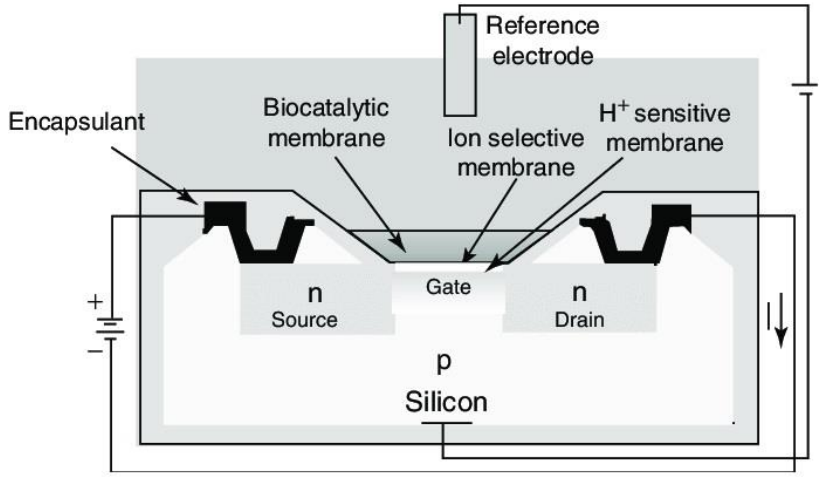

Figure 1.5: Potentiometric Biosensor [21] .

1.5.4 Optical Biosensors

Optical detection biosensors are the most diverse class of biosensors because they can be used for many different types of spectroscopy, such as absorption, fluorescence, phosphorescence, Raman, SERS, refraction, and dispersion spectrometry. In addition, these spectroscopic methods can all measure different properties, such as energy, polarization, amplitude, decay time, and/or phase. Amplitude is the most commonly measured as it can easily be correlated to the concentration of the analyte of interest [22]. In optical biosensors, the optical fibers allow detection of analyte on the basis of absorption, fluorescence or light scattering. Since they are non-electrical, optical biosensors have the advantages of lending themselves to in vivo applications and allowing multiple analyses to be detected by using different monitoring wavelengths. The figure below shows an Optical Biosensor.

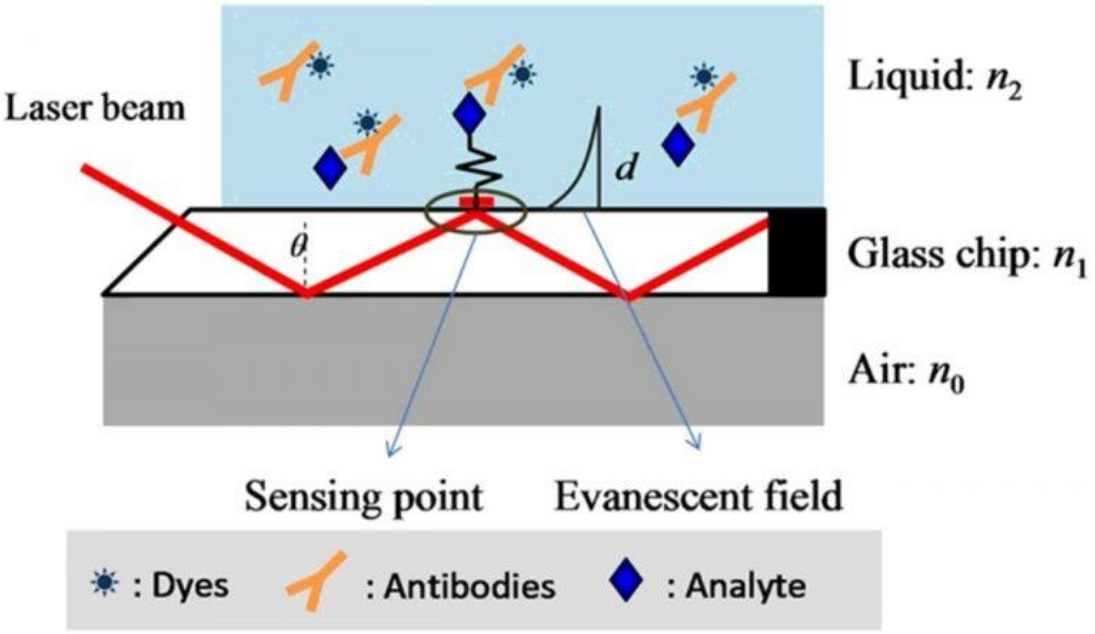

Figure 1.6: Optical Biosensor [23] .

1.5.5 Acoustic Biosensors

Electro acoustic devices used in biosensors are based on the detection of a change of mass density, elastic, viscoelastic, electric, or dielectric properties of a membrane made of chemically interactive materials in contact with a piezoelectric material. Bulk acoustic wave (BAW) and surface acoustic wave (SAW) propagation transducers are commonly used. In the first, a crystal resonator, usually quartz is connected to an amplifier to form an oscillator whose resonant frequency is a function of the properties of two membranes attached to it.

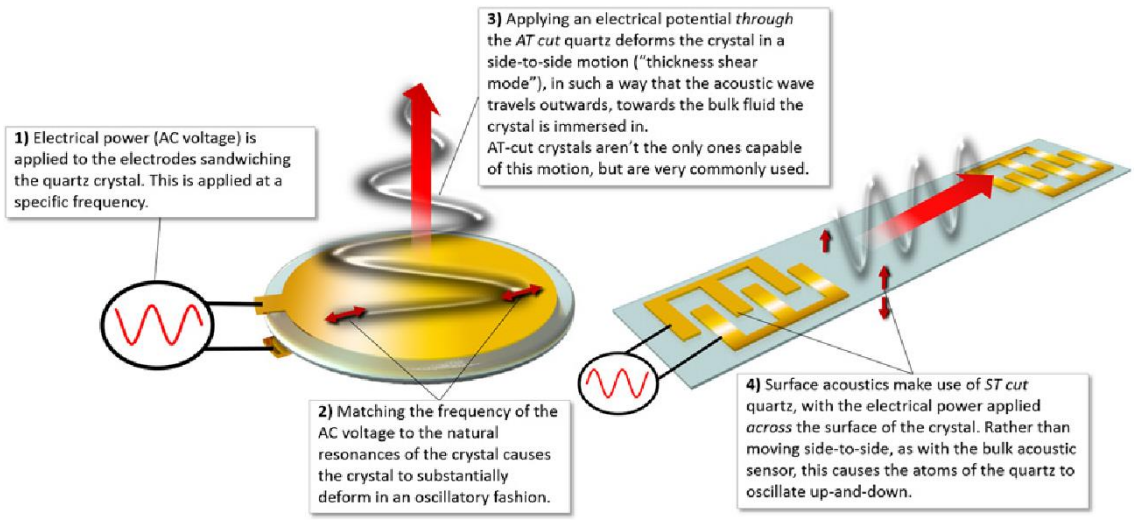

Figure 1.7: Graphic depicting in general terms the processes for the generation of surface and bulk acoustic waves [24] .

1.5.6 Bimolecular sensor

A biomolecule is very interested field part of bio sensing and nanotechnology and simulation COMSOL Multiphysics, is any molecule that is produced by an income organism, including many types of bimolecular large macromolecules such as proteins, DNA and nucleic acids, as well as small molecules such as metabolites, and natural products. In this review they present in Bimolecular detection has become very clear popularly employed in biomedical diagnostics, environmental monitor, forensic and civil defense. COMSOL Multiphysics simulation start in this field of Biosensors are chiefly interesting since they due can compared between high sensitivity of optoelectronic transducers and the high selectivity of bio molecule recognition, which successfully physical and chemical properties. In other hand, nanotechnology give high develop to offers unprecedented opportunities for make the design of highly sensitive and selective bio sensing devices. Can see In the Metal nanoparticles are particularly and use fulin this regard due to their different properties [17].

1.5.7 Enzymatic Biosensor

An enzymatic biosensor comprises of an enzyme, which recognizes and then reacts with the target analyte producing a chemical signal, a transducer, which produces a physical signal out of that chemical one, and an electronic amplifier, which conditions and then amplifies the signal. [25]

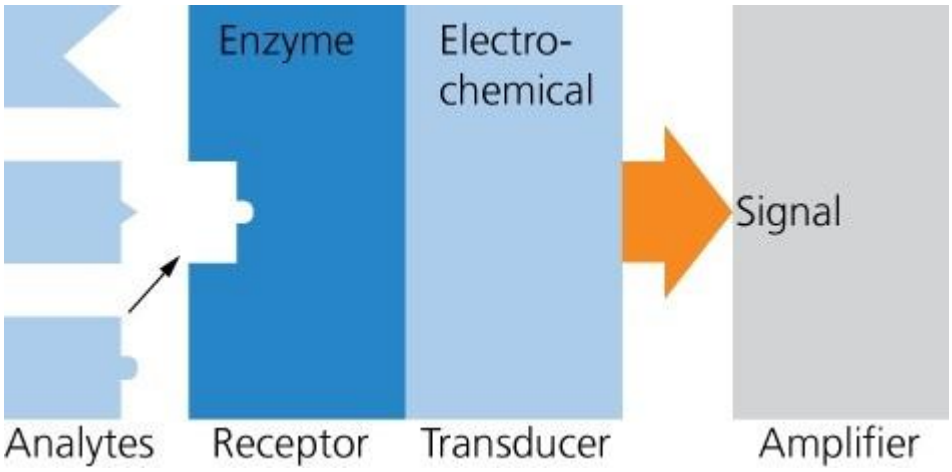

Figure 1.8: General structure of a biosensor. Sensors by IST AG use enzymes to detect glucose, lactate, glutamine and glutamate. The transducer principle is electrochemical, producing a current as output signal [25] .

1.6 PDMS (Polydemethylsiloxane)

1.6.1 Definition

Polydimethylsiloxane called PDMS or dimethicone is a polymer widely used for the fabrication and prototyping of microfluidic chips.

It is a mineral-organic polymer (a structure containing carbon and silicon) of the siloxane family (word derived from silicon, oxygen and alkane). Apart from microfluidics, it is used as a food additive (E900), in shampoos, and as an anti-foaming agent in beverages or in lubricating oils.

For the fabrication of microfluidic devices, PDMS (liquid) mixed with a cross-linking agent is poured into a microstructured mold and heated to obtain an elastomeric replica of the mold (PDMS cross-linked) [26] .

Figure 1.9: Forme of PDMS [26] .

1.6.2 Structure

The chemical formula for PDMS is $CH_3[Si(CH_3)_2O]_nSi(CH_3)_3$, where *n* is the number of repeating monomer $[SiO(CH_3)_2]$ units.[27] Industrial synthesis can begin from dimethyldichlorosilane and water by the following net reaction:

$$n\mathrm{Si(CH_3)_2Cl_2} + (n+1)\mathrm{H_2O} \longrightarrow \mathrm{HO[-Si(CH_3)_2O-]_nH} + 2n\mathrm{HCl}$$

The polymerization reaction evolves hydrochloric acid. For medical and domestic applications, a process was developed in which the chlorine atoms in the silane precursor were replaced with acetate groups. In this case, the polymerization produces acetic acid, which is less chemically aggressive than HCl. As a side-effect, the curing process is also much slower in this case. The acetate is used in consumer applications, such as silicone caulk and adhesives.

1.6.3 PDMS in microfluidics

PDMS is one of the most employed materials to mold microfluidic devices.
We describe here the fabrication of a microfluidic chip by soft-lithography methods: [28]

(1) The molding step allows mass-production of microfluidic chips from a mold.

(2) A mixture of PDMS (liquid) and crosslinking agent (to cure the PDMS) is poured into the mold and heated at high temperature.

(3) Once the PDMS is hardened, it can be taken off the mold. We obtain a replica of the micro-channels on the PDMS block.

(4) To allow the injection of fluids for future experiments, the inputs and outputs of the microfluidic device are punched with a PDMS puncher the size of the future connection tubes.
(5) Finally, the face of the block of PDMS with micro-channels and the glass slide are treated with plasma.

(6) The plasma treatment allows PDMS and glass bonding to close the microfluidic chip. The chip is now ready to be connected to microfluidic reservoirs and pumps using microfluidic tubing. Tygon tubing and Teflon tubing are the most commonly used tubings on microfluidic setups.

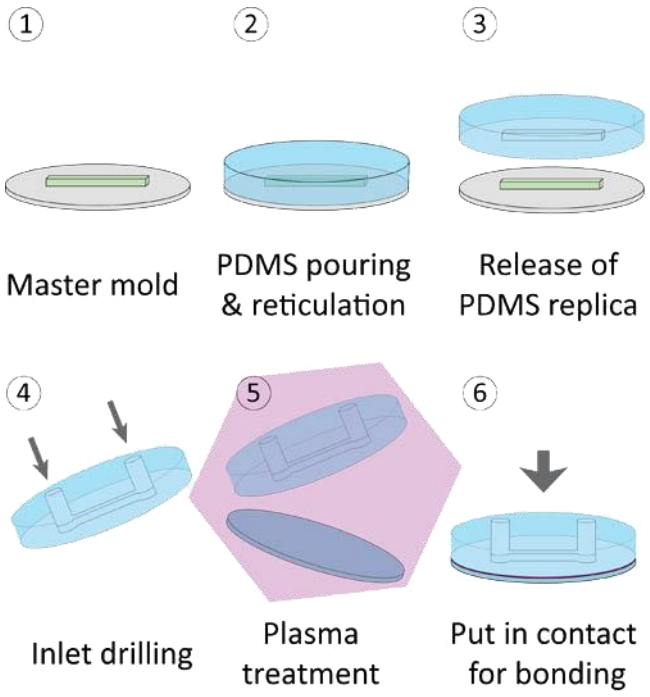

Figure 1.10: Process molding single layer PDMS device [28].

1.6.4 Why we use PDMS for microfluidic device fabrication?

It is transparent at optical frequencies (240 nm – 1100 nm), which facilitates the observation of contents in micro-channels visually or through a microscope.

It has a low autofluorescence [29] It is considered as **bio-compatible** (with some restrictions). The PDMS bonds tightly to glass or another PDMS layer with a **simple plasma treatment**. This allows the production of multilayers PDMS devices to take advantage of the technological possibilities offered by glass substrates, such as the use of metal deposition, oxide deposition or surface functionalization.

PDMS, during cross-linking, can be coated with a controlled thickness on a substrate using a simple spincoat. This allows the fabrication of multilayer devices and the integration of micro valves. It is deformable, which allows the integration of microfluidic valves using the deformation of PDMS micro-channels, the easy connection of leak-proof fluidic connections and its use to detect very low forces like biomechanics interactions from cells. It is inexpensive compared to previously used materials (e.g. silicon).

The PDMS is also easy to mold, because, even when mixed with the cross-linking agent, it remains liquid at room temperature for many hours. The PDMS can mold structures at high resolutions. With some optimization, it is possible to mold structures of a few nanometers [30]. It is gas permeable. It enables cell culture by controlling the amount of gas through PDMS or dead-end channels filling (residual air bubbles under liquid pressure may escape through PDMS to balance atmospheric pressure).

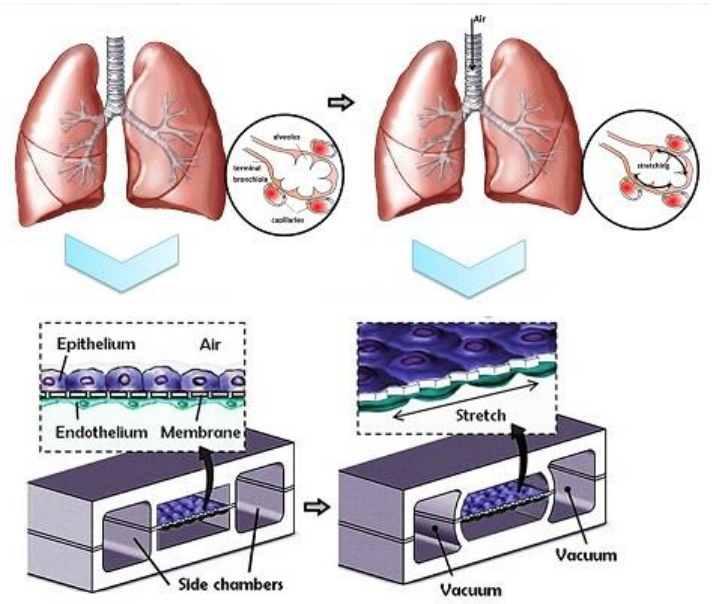

Figure 1.11: Lung on a chip resized [30].

1.6.4.1 PDMS issues for microfluidic applications

It is almost **impossible to perform metal and dielectric deposition on PDMS**. This severely limits the integration of electrodes and resistors. Nevertheless, this problem is minimized by the fact that PDMS easily bonds to glass slides using a plasma treatment, even if large metal areas can prevent a good bonding. Thus, the various thin metal layers or dielectric depositions can be performed on glass slides.

PDMS ages, therefore after a few years the mechanical properties of this material can change. It adsorbs **hydrophobic molecules and can release some molecules** from a bad cross-linking into the liquid and this can be a problem for some biological studies in PDMS microfluidic devices. PDMS is permeable to water vapor which makes **evaporation** in PDMS device hard to control. PDMS is **sensitive to the exposure to some chemicals** (see below). [26]

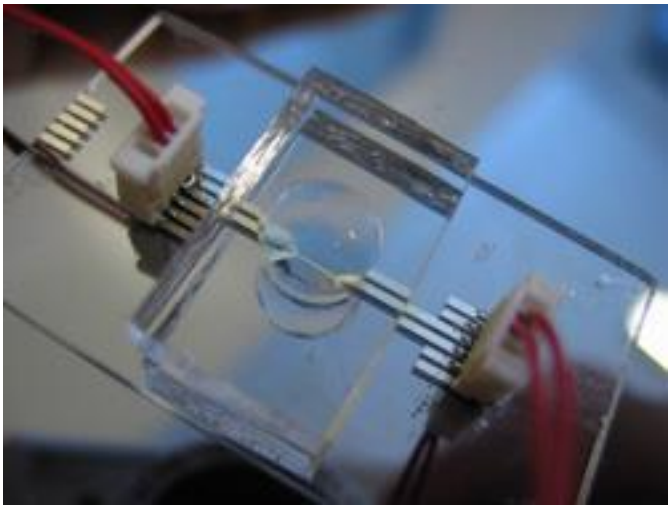

Figure 1.12: Electrodes deposited on glass to be integrated in the Pdms microfluidic chip[26].

1.6.5 Different PDMS used in microfluidic

PDMS is used to fabricate microfluidic devices (single layer and bilayer) and micro-imprint stamps. Two types of PDMS are commonly used by researchers for these applications: PDMS RTV-615 and PDMS Sylgard 184. The exact composition of these two PDMS is… kept secret. However, the experience of researchers can help choosing the most suitable PDMS for an application [31]:

1) PDMS RTV-615

The preferred PDMS of S. Quake (Co-inventor of the microfluidic valve).

The most robust and convenient to bond bilayer microfluidic devices.

It has the reputation for being dirty. (For example, Fluidigm has discarded 90% of the RTV-615 they received).

There are variabilities in plasma bond strength between different batches. This makes it necessary to adjust the bonding parameters with each purchase.

2) PDMS Sylgard 184 (Dow Corning)

- The cleaner PDMS.
- This PDMS is less often used for multilayers chip.
- It makes the bonding more difficult between two PDMS layers.
- It generates more failures during the device fabrication.
- This PDMS is most often used for mammalian cell cultures in microfluidic chips.

Figure 1.13 : PDMS microfluidic chip .

CHAPTER TWO

IMMOBILIZED ENZYME

2.1 Introduction

The use of enzymes is increasingly used in the industrial sectors For example, the agri-food industry uses them to remedy natural deficiencies ingredients, to develop manufacturing processes. The detergent industry (90% of detergents contain enzymes), the medical industry (therapeutic treatments) also use of these active biomolecules [32].

The study of biosensors based on enzyme encapsulation in matrices inorganic is a research area in strong development. Indeed, the enzymes are more in addition used for molecular detection in the fields of the environment and the medicine. However, their low stability and high cost require their immobilization in porous and protective solid matrices. The development of these "biomaterials “Functional must meet a strict specifications imposing in particular a density high level of immobilized biomolecules in the inorganic matrix, good accessibility of the substrate, the conservation of biological activity over time and stability operational and storage.

Many immobilization methods have been developed for the realization of electrochemical biosensors such as covalent grafting or inclusion in matrices Organic and inorganic open framing. Hybrid bio-inorganic materials constitute a new class of materials at the interface between biology and materials science.

2.2 Enzyme Definition

Enzymes are biological catalysts of living organisms, they are macromolecules of protein and chiral nature [32]. They consist of several α-acids amines of the L series joined together by a bond formed by condensation between the group carboxyl of one amino acid and the amino group of another amino acid to form a amide bond. Enzymes are therefore high molecular weight polypeptides between 10 to 1,000 kDa. The order in which the amino acids are arranged constitutes what is called the primary structure of enzymes (Figure2.1).

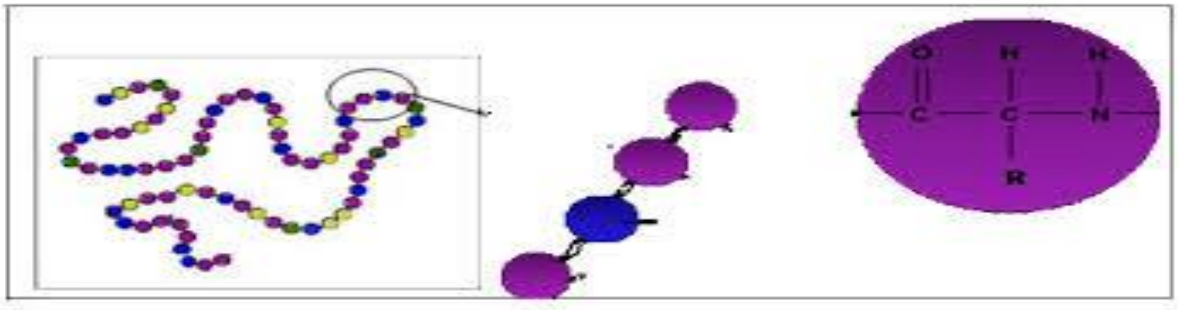

Figure 2.1: The primary structure of enzymes

These proteins will tend to fold on themselves to form secondary arrangements mainly α-helices and β-sheets (Figure2.2); this structure is stabilized through the generation of hydrogen bonds.

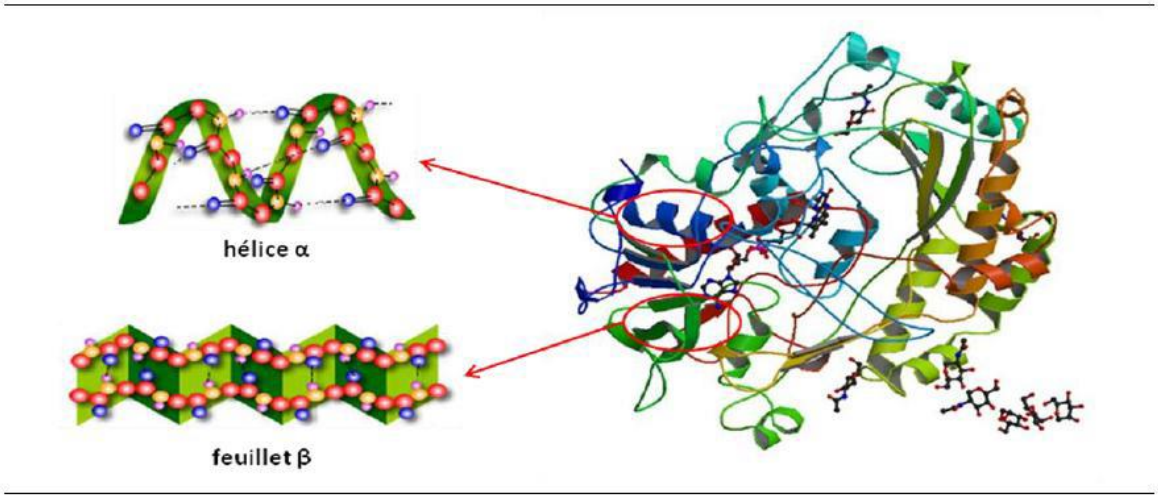

Figure 2.2: Schematic representation of the secondary structure of a protein [32].

The arrangement of these secondary structures with respect to one another forms.A tertiary structure which will be stabilized by disulfide bridges (Figure 2.3).

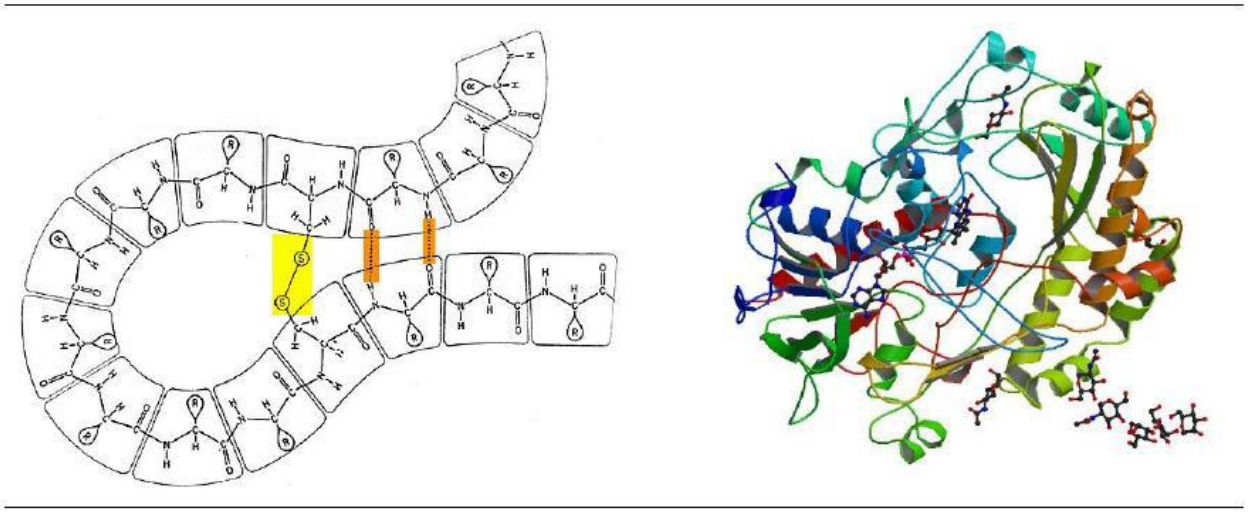

Figure 2.3: Schematic representation of the tertiary structure of a protein [33].

A quaternary structure can even be described for very large enzymes (Figure 2.4). This three-dimensional structure of the enzyme will give it its specificity allowing it to recognize a particular substrate via a distinct region of the enzyme, called the site active.

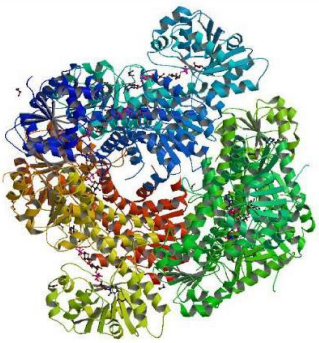

Figure 2.4: Schematic representation of the quaternary structure of a protein (the glucose dehydrogenase) [34].

2.2.1 Enzyme Activity

An enzyme is [33] .a protein that acts as a catalyst in a chemical reaction, that is to say, it increases the reaction rates. The activation energy of the reaction is then lowered. Each enzyme specifically "recognizes" one or more molecules according to a key-lock complementarity principle thanks to recognition and Surface They are specific catalysts since they can only participate in definite reactions. At the end of the process, the enzyme regains its structure. It should be noted that enzymes participate in reactions taking place in living organisms. Thereby, the enzyme operates under mild conditions of temperature, pH and pressure. The catalytic reaction takes place at the catalytic site called the active site of the enzyme, Located in the protein pocket, cavity with specific structural and chemical characteristics. The active site of some proteins called apoenzyme can only be effective by via a cofactor. The latter of the metallic type (copper, zinc, manganese) or organic type (coenzyme) binds to the catalytic region to promote chemical reaction and also contribute to the stability of the enzyme. The reaction can only take place by the combination of the apoenzyme with the cofactor. The target chemical, named substrate(S), binds to the enzyme (E) at the active site that allows the reaction. It then forms a intermediate complex ES. After reaction, the product (P) is released and the enzyme regenerated. This process is done thousands of times per second:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P$$

2.2.2 Enzyme Classifcation

The catalytic power of enzymes makes it possible to produce new substances and energy, essential for the proper functioning of living organisms. It's according to their catalytic activity that these are classified. A nomenclature has been proposed by the Commission of Enzymes of the International Union of Biochemistry dividing enzymes into six large classes (Table2.1):

First E.C digit	Enzyme class	Reaction Type
1	Oxedureductases	Oxidation/Reduction
2	Transferases	Atom/group transfer (excluding other classes)
3	Hydrolases	Hydrolysis
4	Lyases	Group removal
5	Isomerases	Isomerization
6	Ligases	Joining of molecules linked to the brekage of a pyrophosphate bond

Table2.1: Enzyme classification [33].

Each class is divided into subclass and each subclass into subclass. A "number" of classification is associated with each enzyme and is called "EC number". He is This is as follows: EC [class number]. [subclass number]. [sub-subclass number]. [individual serial number in the subclass]. Take the example of glucose oxidase: EC 1.1.3.4. This figure is explained below:

EC 1: Oxidoreductase.

EC 1.1: Acting on the CH-OH group of the donor.

EC 1.1.3: With oxygen as acceptor.

The last digit is the individual number of the enzyme. It is important to define the involvement of the enzyme during the chemical reaction. The amount of enzyme present or used in a process is difficult to determine in terms of absolute (for example: gram) since an enzyme has variable proportions of active and inactive material. Indeed, the reaction rate is related to the enzymatic activity which is defines as a unit / mass of protein. This unit releases one micromole of product per minute under optimum conditions in terms of pH, ionic strength and temperature.

2.3 Immobilized Enzyme what interest?

The development of new analytical methods for research and industry more and more uses the use of specific enzymes. However the cost of production enzymes remain prohibitive. In fact, they must first be extracted from biological media then purify them. In addition, because of their often high solubility, enzymes can be contaminated with the reaction product which is in the same phase. The enzyme is dirty and its purification entails new expenses. Moreover with time enzymes [35] denature and therefore do not work as effectively. It is necessary to replace them after several cycles of use, but this involves additional expenses. It is therefore essential to find a method to stabilize the enzymes against their denaturation. The immobilization of enzymes in solid matrices or in gels allows the separation of the protein and the reaction product, in two different phases, preventing the contamination with the product. This process then allows the reuse of the biomolecule. The most commonly used immobilization materials are chemically inert materials, insoluble and rendering the enzyme insoluble. These are more particularly matrices polymeric and inorganic mono, bi, or three-dimensional.

2.4 Different Methods of Immobilization

Enzymes have a great interest in the field of biocatalysis. However, as we have mentioned, their cost and limited stability over time are factors limiting their industrial use. The immobilization of the enzymes which makes it possible to stabilize these during their use, to be able to reuse them and to separate the enzyme from the products of the enzymatic reaction. In 1916, Nelson and Griffin will be the first to demonstrate that an enzyme, in the occurrence of invertase, maintains its catalytic activity even after being immobilized by adsorption on activated charcoal. This technique will experience a real boom in from the 1950s with the first applications in various fields. It exists different immobilization techniques that can be both chemical and physical. We may include five commonly used methods, each of which has its advantages and their disadvantages.

- ✓ Adsorption on an inert support (physical adsorption).
- ✓ By covalent bond.

1) On media.

2) Reticulation (without support).

- ✓ Encapsulation or inclusion.
- ✓ Immobilization by nanostructuration of molecular films (Technique Langmuir Blodgett).

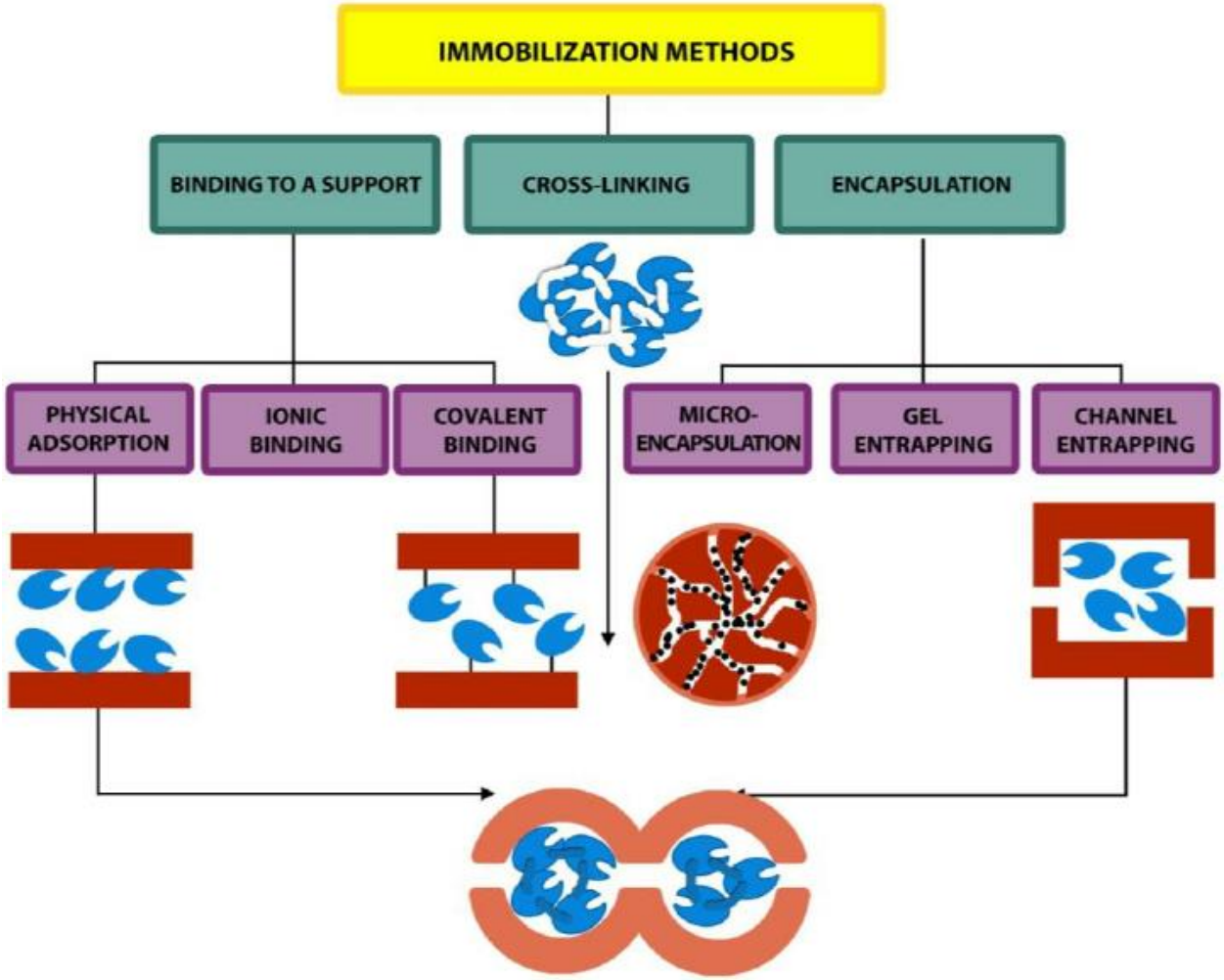

Figure 2.5: Immobilization Methods [36].

2.5 Immobilization by adsorption

Enzyme adsorption on insoluble matrices is the simplest method immobilization. The procedure involves incubation of the protein with the adsorbent in appropriate conditions (pH, ionic strength, temperature). Hybrid material is recovered after filtration or centrifugation. The interactions that exist between the enzyme and the adsorbent, are weak interactions of the electrostatic or van der Waals type. However, the process adsorption is reversible and may lead under certain conditions of use to the desorption of the biomolecule and therefore the alteration of the material. However different modes immobilization, adsorption is the method that induces the least modification of the conformation of the active enzyme, which is why it is the preferred technique for immobilization of enzymes.

2.5.1 The supports used in this method

a-Organic supports:

Includes polysaccharides such as cellulose acetate, cellulose nitrate, dextran, agarose and polymers such as polystyrene, polypropylene.

b-Inorganic supports (Mineral supports):

Are generally more stable, resistant to chemicals and bacteria. Active materials can be:

- ✓ The clays
- ✓ Porous glass and porous silica

c-Other supports :

- ✓ Nylon
- ✓ Ceramic oxide
- ✓ Collagen and activated charcoal

2.5.2 Parameters that influence adsorption

- ✓ The enzyme concentration
- ✓ The contact Time
- ✓ Composition of the environment
- ✓ Temperature

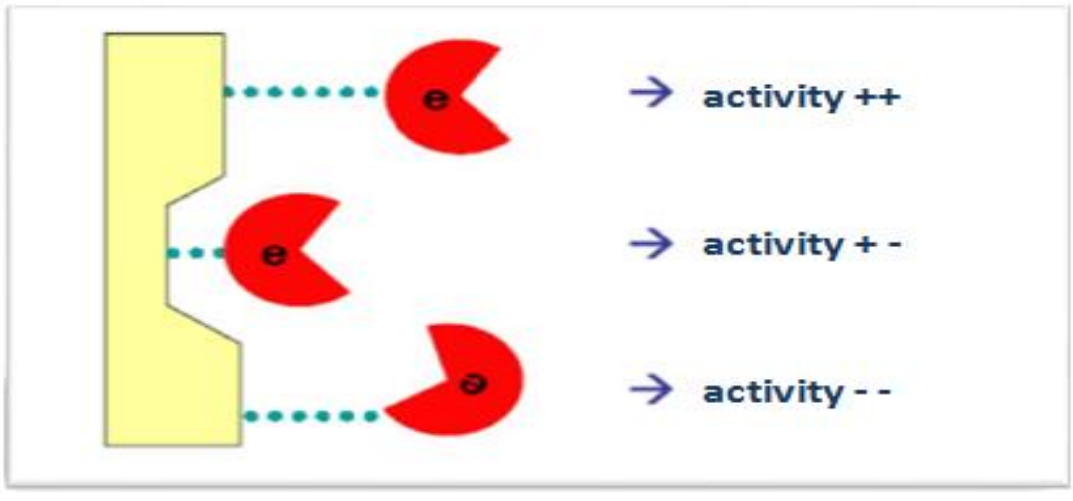

Figure 2.6: Orientation possibility [35].

a-Advantages :

- ✓ Adsorption is easy to implement, it is sufficient to put the enzyme into contact with the support.
- ✓ Possibility of regenerating the enzyme-support complexes (if the enzyme loses its activity during its operation, it is possible to replace it with a preparation active).
- ✓ Fast fixing of the support and simple and non-denaturing immobilization.

b-Disadvantages:

- ✓ The fragility of fixation (the enzymes can easily be desorbed under the action of pH variation, temperature.
- ✓ The orientation of the enzyme and poor accessibility to the active site.

2.6 Covalent bonding

Enzyme immobilization by covalent binding is one of the most widely used methods, in which stable complexes between functional groups on enzyme molecules and a support matrix are formed through covalent bondings. The functional group present on enzyme, through which a covalent bond with support could be established, should be non-essential for enzymatic activity which usually involves binding via the side chains of lysine (ε-amino group), cysteine (thiol group) and aspartic and glutamic acids (carboxylic group). The enzyme functional groups that could be utilized in covalent coupling include: Amino group, carboxylic group, phenolic group, sulfhydryl group, thiol group, imidazole group, indole group and hydroxyl group [37]. The binding procedure of enzyme to the solid support generally goes through two stages: (1) activation of the surface using linker molecules such as glutaraldehyde or carbodiimide and (2) enzyme covalent coupling to the activated support. Linker molecules are multifunctional reagents (glutaraldehyde or carbodiimide) act as the bridge between surface and enzyme via covalent bonding. While the first group matches the immobilization surface and forms a so-called self-assembled monolayer (SAM), the second ground bound to preactivated support then forms a covalent bond with the enzyme. Different linkers are used for different surfaces (inorganic material, natural or synthetic polymer, membranes) and immobilization protocols (directly onto the transducer surface or onto a thin membrane fixed onto the transducer).

Covalent immobilization provides strong bindings between enzymes and support matrix and therefore little leakage of enzyme from the support may occur. In addition, high uniformity of the SAM layer and good control of the immobilized enzyme amount are the

other advantages. In covalent attachment, there is a high risk of enzyme denaturization when most enzymes must go through chemical modifications to possess functional group. In addition, the method requires high volume of bioreagent but only small amounts of enzymes may be immobilized (~0.02 grams per gram of matrix). The immobilization procedure largely increases enzyme stability but decreases enzyme activity in affinity reaction and is poorly reproducible [38]. In comparison to adsorption, covalent bonding requires longer incubation time, since the formation of the SAM and the subsequent linkage of the enzymes to it take several hours. The process is also more complex and care has to be taken to ensure chemical purity so that the SAM is obtained in high homogeneity. The most used procedures to covalently immobilize enzyme on functionalized surface (through the activations of carboxylic group and amino group) are briefly described below.

2.6.1 Activation of carboxylic groups

A carbodiimide is a functional group (formula RN=C=NR) which allows the binding between the carboxyl groups (-COOH) of a support and the amino function ($-NH_2$) of an enzyme. In order to improve immobilization efficiency, N-hydroxysuccinimide (NHS) could be associated to carbodiimide prior to enzyme covalent coupling step.

2.6.2 Activation of amino groups

The binding between an amine functionalized support and carboxyl functionalized enzyme could also be done with carbodiimides. Alternatively, glutaraldehyde could be used as the activating agent for enzyme immobilization. Firstly, Schiff-base reaction occurs between amine functionalized support and an aldehyde group of glutaraldehyde and then, the second aldehyde group of glutaraldehyde covalently bind to an amine functionalized enzyme.

2.6.3 Chemisorption

The principle of this immobilization method based on a strong affinity and semi-covalent bond between thiol group (-SH) and gold substrates (Au). Thus, thiol-containing enzymes, such as oxidoreductases and isomerases which contain double-catalytic site cysteine residues, could be immobilized on gold surface via the thiol groups of their amino acid residues. These thiol-containing enzymes are either in native forms or obtained through chemically modification or genetic engineering techniques, in order to provide them with reactive thiol groups. A detailed paper on immobilization of enzyme via their thiol group could be found here [39]. Alternatively, thiol containing enzymes can be immobilized onto supports, which fixed with reactive disulfides or

disulfide oxides, through a thiol-containing bifunctional linker which, on one end, forms disulfide bonds (S–S) to the surface, and on the other end, provides N-hydroxysuccinimide (NHS) groups that can react with the free amino groups on the enzyme.

2.7 Entrapment

In entrapment immobilization, enzyme is not directly attached to the support surface but entrapped within a polymeric network which allows only the traverse of substrate and products but retains the enzyme hence enzyme diffusion is constrained. Entrapment immobilization process is conducted through two steps: (1) mixing enzyme into a monomer solution, followed by (2) polymerization of monomer solution by a chemical reaction or changing experimental conditions. As an enzyme is physically confined within a polymer lattice network, the enzyme does not chemically interact with the entrapping polymer. The method thus could improve enzyme stability and minimize enzyme leaching and denaturation. Another advantage of the method is the capability to optimize microenvironment for the enzyme by modifying the encapsulating material to have the optimal pH, polarity or amphilicity. However, a limitation of the method is the mass transfer resistance occurred as polymerization extension tends to increase the gel matrix thickness, substrate for this reason cannot diffuse deep into the gel matrix to reach the enzyme active site. Furthermore, the entrapped enzymes are likely to suffer from leakage if the pores size of the support matrix is too large. The method also has low enzyme loading capacity and the support material could be corrupted as effects of polymerization. There is a variety of procedures used in entrapment immobilization depending on type of entrapment such as electropolymerization, photopolymerization, sol-gel process for lattice or fiber type and microencapsulation for microcapsule type [40].

2.7.1 Electrochemical polymerization

Electrochemical polymerization (or electropolymerization) is a simple approach in which an appropriate potential or current is applied into a solution containing both enzyme and monomer molecules. The oxidization or reduction reactions of monomers occurred in the solution at electrode surface could then generate reactive radical species which couple together and finally form an adherent polymer at the electrode surface. Enzyme molecules that are present in the solution close by the electrode surface are trapped inside the growing polymer network as polymerization process propagates. The first step in the polymerization process is the oxidation of the monomer

to generate a radical cation which then could either react with a neutral monomer or with another similar radical to form a dimer. The formed dimers then undergo further oxidation process and coupling reactions to generate oligomers and finally produce an insoluble polymer deposited on electrode surface. Most of electropolymerized films used for enzyme immobilization are electronically conducting polymers such as polyaniline, polypyrrole or polythiophene, pyrroles, thiophenes and polyindole. In addition, other materials such as redox conductors as in the case of metal poly(pyridine) complexes and non-conducting (insulating) polymer, as in the case of phenols, 1, 2-diaminobenzene are also applicable for electropolymerization. However, electropolymerized films of conducting polymers have been predominantly employed in various sensor types. The distinct advantages of conducting polymers over other materials is the conductivity which helps control the deposition site and thickness of the polymer films easily because the continuing growth of the polymer thickness is exclusively done on electrode surface and driven electrochemically by the applied potential and propagation time. In comparison to manual deposition, electrochemical deposition by mean of polymerization has better controls over the homogeneity and thickness of the polymer film because the homogeneity could be evaluated from the formation of a diffusion barrier over the film and the thickness could be measured by the charge transferred during film formation. Other parameters that could affect the nature and morphology of the polymer film are the choice of solvent, counter-ion, and conditions used in the electrochemical polymerization such as temperature, monomer concentration and the electrolyte chain length of the polymer. Electrochemical polymerization offers a simple one-step method which could produce homogeneous films by an easy control of applied potential. On the electrode, high enzyme activity is retained because there is no interaction between enzyme and monomer during the polymerization process which propagates exclusively on the electrode surface. However, there are some criteria that one should follow for a reproducible immobilization of enzyme as follows: The polymerization process should be carried out in an oxygen-free environment with proper polymerization solution. The change of pH and charge of the polymer due to protons liberation during the polymerization reaction may affect enzyme activity and should be compensated by incorporation of anions from the electrolyte. The drawbacks of this method is the requirement of high concentrations of monomer (0.05–0.5M), enzyme (0.2–3.5 mg ml^{-1}) and film deterioration resulted from overoxidation process which happens due to monomer depletion in electrode surrounding solution [40] .

2.7.2 Photopolymerization

In photopolymerization process-based enzyme immobilization the use of liquid, photopolymers (radiation curable resins) and enzyme solution are required. The photopolymerization reactions are chain-growth polymerizations which are initiated when the photopolymers exposed to light in the ultraviolet or visible region of the electromagnetic spectrum. Upon light exposure, these photopolymers undergo chemical reactions for cross-linking of molecules resulting in the hardening of the material. The reactant monomer may absorb light either directly or through an energy transfer from a photosensitizer. In general a photopolymerization process goes through 4 stages of initiation, propagation, termination and chain transfer steps. Also, the polymerization process has been used to entrap enzymes for poly(vinyl alcohol)-bearing styrylpyridinium groups (PVASbQ), a soluble pre-polymer bearing photo-crosslinkable groups, which has largely been used to entrap enzymes since its first synthesis [41–42]. In the propagation of photopolymerization reactions, light irradiation is required to triggers additional cross-linking reactions between comonomers to form oligomers and finally generate an insoluble polymer.

2.7.3 Sol-gel process

The sol-gel process is based on the ability to form metaloxide, silica, and organosiloxane matrices of defined porosity by the reaction of organic precursors at room temperature [43]. There are two generic methods of the sol-gel technique depending on the types of starting materials (precursors) used: colloidal method, and polymeric (or alkoxide) route. In enzyme immobilization the latter method is commonly employed. The route involves 2 following steps: (1) Suspending or dissolving the metal alkoxide precursor(s) such as tetramethoxysilane or methyltrimethoxysilane in a suitable liquid (acidic pH in the presence of water) for hydrolyzation to produce silanol (Si-OH) groups. (2) The hydrolized precursor is then activated by the addition of a base (such as potassium hydroxide) to initiate condensation reactions between silanol moieties resulting in the formation of siloxane (Si-O-Si) polymers. As the network grows and ages with time and temperature, the viscosity of the liquid increases at an exponential rate until gelation occurs. As a result, a matrix is created in which the enzyme molecules are enclosed within the network [44–45]. Sol-gel formation is a popular immobilization method that results in a stable nanoporous material where enzyme activity is preserved and biosensor sensitivity is enhanced owing to high encapsulation concentration and mild immobilization conditions. However the method may suffer from extra cost of

precursors and matrix inhomogeneity due to fracture during gelation drying and precipitation of oxides during sol formation.

2.7.4 Micro-encapsulation

Immobilization by encapsulation represents an entrapment method in which enzymes are enclosed in a spherical semi-permeable membrane. The membrane may be polymeric, lipoidal, lipoprotein based or non-ionic in nature. In general, there are two methods for microencapsulation: (1) Coacervation (or phase separation) in which enzyme microdroplets are separate out in a water immiscible solvent and (2) Interfacial polymerization in which a monomer is made to be polymerized at the interface of two immiscible substances (a hydrophobic monomer and another monomer which is dispersed in a water immiscible solvent). This polymerization process results in the occlusion of enzyme within the polymeric membrane. The immobilization principle is based on the difference in size of enzyme and substrate or product molecules in comparison with membrane pore size. The membrane allows small sized molecules such as substrate/product to diffuse in and out of the membrane while refrains larger-size enzyme molecules within. Therefore, enzyme molecules are confined by the membrane and are free floating inside the capsule. The encapsulation method offers the integrity of enzyme structure and activity because enzymes are protected from medium conditions thus the risk of enzyme leakage is minimal. In addition, multi-enzyme system could be developed by trapping more than one enzyme inside the membrane [46]. One disadvantage of the method is the requirement for accurate control of membrane pore size to prohibit enzyme leakage. Therefore, the membrane porosity must be specifically adjusted according to different molecule size of enzyme and substrate. This technique hence is inapplicable for reactions in which substrate and enzyme molecules are of similar diameters or reactions that substrate molecular size is too large.

2.8 Cross-linking

Enzyme immobilization by cross-linking is an irreversible method performed by the formation of intermolecular cross-linkages between the enzyme molecules by covalent bonds. The process is carried out with the assistance of a multifunctional reagent which acts as linkers to connect enzyme molecules into three dimensional cross linked aggregates. The immobilized enzyme is present in the reaction mixture and not bound to any support. There are two approaches in cross linking immobilization which are the uses of cross linking enzyme aggregate (CLEA), and cross linking enzyme crystal

(CLEC). Both methods require the use of a cross linking agent such as glutaraldehyde to cross-links enzyme molecules via the reactions of the free amino groups of lysine residues on the reactive site of neighboring enzyme molecules. In CLEC-based method, glutaraldehyde is added to cross-link enzyme crystals after crystallization. Enzymes immobilized by CLEC usually possesses significant improvements in mechanical properties thus immobilized enzyme in CLECs are usually stable and having higher efficiency than the untreated forms. CLEA is an improved version of CLEC production which could work in aqueous solutions while CLEC requires the formation of crystals. In CLEA-based method, the addition of salts, organic solvents or non-ionic polymers results in the formation of enzyme aggregates which retain enzyme catalytic properties.

Immobilization by crosslinking is a simple method which based on the strong chemical binding of enzyme biomolecules thus enzyme leakage is minimal. Another advantage of the method is the possibility to adjust microenvironment for enzyme by using suitable stabilizing agents through surface complementarity which helps increase stability [47]. However, the use of glutaraldehyde could result in severe enzyme modifications and possibly lead to enzyme conformational changes and loss of activity. For this reason, inert proteins like gelatin, bovine serum albumin (BSA) may be added during the immobilization process to minimize this drastic modification of enzymes [48].

2.9 Immobilization of enzyme on microchannel surface

Methods for enzyme immobilization on the microchannel surface have also been developed because they can take advantage of the larger surface area of microreaction systems without pressure increases.

Physical immobilization is an easy way to immobilize molecules. In microchannel systems, a biotin-avidin system has mainly been used to immobilize enzymes. The biotinylated polylysine was physically immobilized on a glass surface to immobilize streptavidin-conjugated alkaline phosphatase [49]. This microreactor was used for rapid determination of enzyme kinetics. Biotinylated lipid bilayer [50] and partial biotinylation by photo patterning on fibrinogen [51] were also used for immobilization. However, these methods are not suitable for long-term use because of their instability. Also, applications are limited to streptavidin-conjugated enzymes.

The introduction of a functional group on the microchannel surface was used for covalent crosslinking. A trypsin-immobilized microreactor was prepared by modification with 3- aminopropylsilane and glutaraldehyde using the classical method [52]. Although this immobilization method is easy, fabrication of complex microstructures is required to achieve high performance. Our group developed a modified sol-gel technique to form

nanostructures on a silica microchannel surface [53]. This method modifies the microchannel surface with polymerized copolymer of 3-aminopropylsilane/methylsilane. Using this method, increased surface area was obtained. At least 10 times more enzymes can be immobilized on these nanostructures by covalent cross-linking through amide-bond formation, disulfide or His-tag, by modifying succinate spacer, compared with single layer immobilization [54-56]. A microreactor with immobilized cucumisin on the nanostructured surface could process substrate 15 times faster than the corresponding batchwise reaction [55].

Similar surface modification methods employing sol-gel techniques were also developed [57]. A PMMA surface was modified with a copolymer of butyl methacrylate/γ-(methylacryloxy)- propyltrimethoxysilicane and silica-sol-gel to immobilize enzymes. Using this method, a trypsinimmobilized microreactor was developed. In addition, a trypsin-encapsulated titania and alumina gel matrix was immobilized through SiOH group formed on a PDMS surface by plasma oxidation [58]. Using this device, digestion time was significantly shortened (ca. 2 s) and the application for highthroughput protein identification was realized. Ji *et al*. developed the layer-by-layer nanozeoliteassembled network to immobilize enzymes in the porous structure formed within zeolite (Figure 1a) [60]. Alternatively, silicone rubber material was used for the preparation of functional nanostructure on the microchannel surface (Figure 1b) [61].

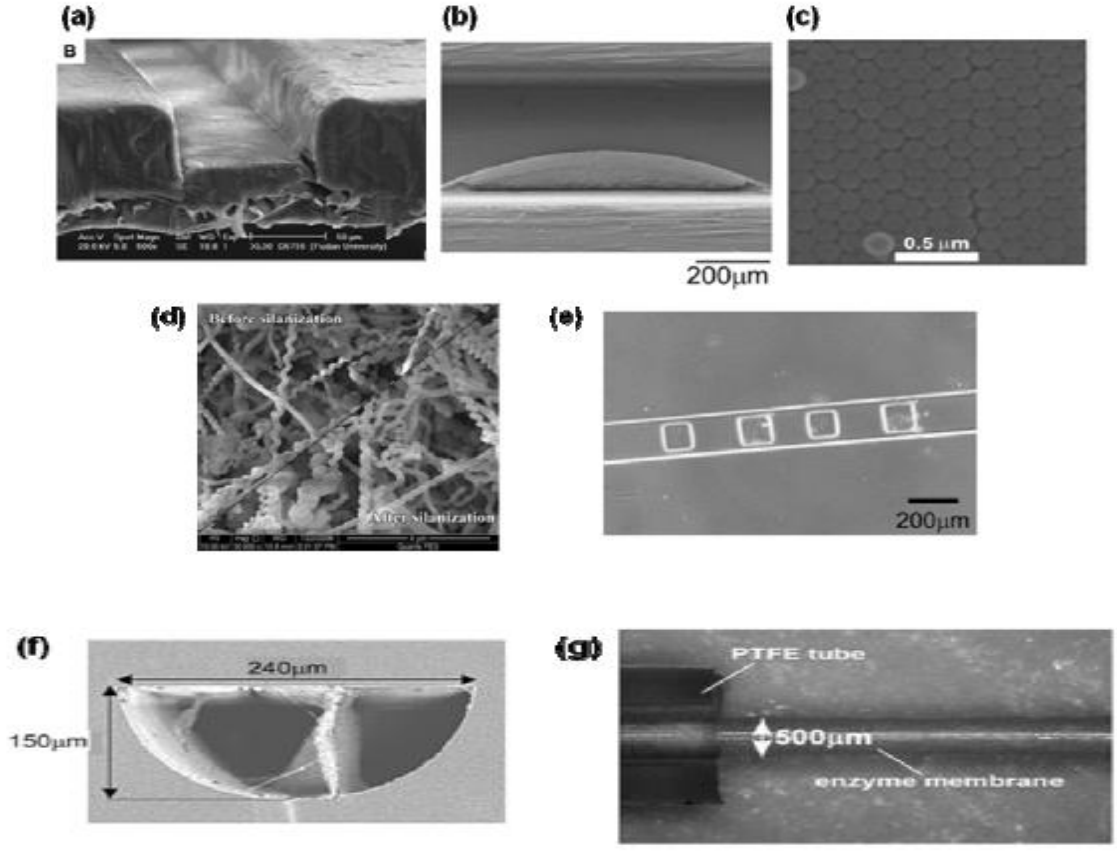

Figure 2.7: Look description below .

Figure 2.7 Images of surface modification and membrane formation techniques for micro enzyme reactor. Modified surface obtained by functionalized microstructure fabricated from layer-by-layer nanozeolite-assembled network (**a**), silicone rubber (**b**), nanoparticle arrangement (**c**), SiO2 nanospring structures (**d**), and hydrogel formation (**e**). Membrane formed within the microchannel can also be used as support for enzyme immobilization. Nylon membrane formed at liquid-liquid interface (**f**), or membrane of cross-linking enzyme aggregate formed at microchannel surface (**g**) was used for immobilization. These images were reproduced with permission from references [59,60 ,62 ,64 ,66 ,67 ,51].

The structure was prepared by micromold fabrication using vinyl-group-containing PDMS and silicic acid, and enzyme immobilization by cross-linking with glutaraldehyde. Using this procedure, a microstructured enzyme reactor with immobilized thermophilic β-glycosidase capable of performing hydrolysis at 80 °C was created.

A particle-arrangement technique was also applied for enzyme immobilization. Silica nanoparticles were immobilized onto the surface using slow evaporation of particle suspension filled-in microchannel (**Figure 2.7c**) [68]. The obtained microchannel was subjected to treatment with 3-aminopropyltriethoxysilane, and immobilization of enzyme was achieved by covalent cross-linking through the amino groups. Although physical stability needs to be improved, a lipase-immobilized microreactor prepared by this method showed 1.5 times faster kinetics than that of microreactor obtained by sol-gel surface modification [61]. This result showed good correlation with the surface area; particle arrangement has approximately 1.5 times larger surface area and could immobilize more enzymes. A SiO2 nanospring structure formed by chemical vapor deposition was also used as immobilization supports.(**Figure 2.7d**) [62]. Photochemistry has been applied to enable selective immobilization of enzymes on the microchannel surface [63]. In the procedure, vinyl azlactone was photografted onto a PEG-coated polymer surface as a reactive monomer and the enzymes were immobilized through their amino groups. This approach was applied for immobilization of horseradish peroxidase. Another approach for efficient enzyme immobilization is polymer coating. Poly(ethylene glycol)based-hydrogels which incorporate alkaline phosphatase was prepared within a microchannel by exposure to UV light (Figure 1e) [64]. This method was also applied to immobilize urease and different enzymes on microchannel surfaces. Overall, these techniques need expensive equipments and/or specialized fabrication skills.

2.10 Conclusion

Advantages that an efficient enzyme immobilization could bring about are the repetitive use of a single batch of enzymes, improved stability, abilities to stop the reaction rapidly by the removal of enzyme from the reaction solution, easy separation of the enzyme from the product and the avoidance of enzyme-product contamination. In addition, multi-enzyme reaction systems for multiple analyte detection could be developed for biosensor applications based on the enzyme immobilization. The choice of immobilization method in biosensors depends on many factors, such as the nature of the biological element, the transducer type, the physicochemical properties of the analyte and the biosensor operating conditions.

CHAPTER THREE

MODELING OF BASIC PHENOMENA (PHYSICS) ENTERING IN OUR BIOSENSOR

3.1 Introduction to the Finite Element Method (FEM)

The description of the laws of physics for space- and time-dependent problems are usually expressed in terms of *partial differential equations* (PDEs). For the vast majority of geometries and problems, these PDEs cannot be solved with analytical methods. Instead, an approximation of the equations can be constructed, typically based upon different types of *discretizations*. These discretization methods approximate the PDEs with *numerical model equations*, which can be solved using numerical methods. The solution to the numerical model equations are, in turn, an approximation of the real solution to the PDEs. The *finite element method* (FEM) is used to compute such approximations [69].

3.2 Comsol multiphysics

3.2.1 History

The COMSOL Group was founded by Mr. Svante Littmarck and Mr. Farhad [70] in Sweden in 1986. It has now grown to United Kingdom, U.S.A, and Finland and so on. Nowadays, The COMSOL Multiphysics software has been widespread used in various domains of science research and engineering calculation, for example, it was used in global numerical simulation. [70; 71].COMSOL Multiphysics is a finite element analysis, solver and Simulation software package for solving various physics and engineering applications. The first version of COMSOL Multiphysics software was published in 1998 by COMSOL group and it was 33 named as Toolbox. At the beginning time, this software is only applied in the field of Structural Mechanics. The COMSOL Multiphysics simulation environment facilitates all steps in the modeling process: defining your geometry, specifying your physics, meshing, solving and then post-processing your results [72].

3.2.2 Introduction

COMSOL Multiphysics is an integrated environment for solving system of time-dependent or stationary second order in space partial differential equations in one, two, and three dimensions. Moreover, such equations may be coupled in an almost arbitrary way. COMSOL Multiphysics provide sophisticated (and convenient) tools for geometric modeling. Therefore, for many standard problems, there exist predefined so-called application modes which act like templates in order to hide much of the complex details of modeling by equations. The application modes make use of the language used in the respective engineering discipline [73]**.**

COMSOL (formerly known as FEMLAB) is a finite element analysis and solver software package for various physics and engineering applications, especially coupled phenomena, or Multiphysics. It includes a complete environment for modeling any physical phenomenon that can be described using ordinary or PDEs. It has become the industry standard for Multiphysics modeling, research, design, and development (COMSOL 2008b; Zimmerman 2006). The software package supports nearly all platforms (e.g., Windows, Mac, Linux, and UNIX). COMSOL allows for building coupled systems of PDEs. The PDEs can be entered directly or using the so-called weak form. COMSOL also offers an extensive and well-managed interface to Math Works MATLAB and its toolboxes for a large variety of programming, preprocessing, and post processing possibilities [74].

COMSOL Multiphysics is a powerful interactive environment for modeling and solving all kinds of scientific and engineering problems based on partial differential equations (PDEs). With this product you can easily extend conventional models for one type of physics into Multiphysics models that solve coupled physics phenomena— and do so simultaneously. Accessing this power does not require an in-depth knowledge of mathematics or numerical analysis. Thanks to the built-in physics modes it is possible to build models by defining the relevant physical quantities—such as material properties, loads, constraints, sources, and fluxes rather than by defining the underlying equations.

3.2.3 PDE Modes

COMSOL Multiphysics internally compiles a set of PDEs representing the entire model. Accessed the power of COMSOL Multiphysics as a standalone product through a flexible graphical user interface, or by script programming in the MATLAB language. As noted, the underlying mathematical structure in COMSOL Multiphysics is a system of partial differential equations. In addition to the physics mode and the modules, these provide three ways of describing PDEs through the following PDE modes:

• Coefficient form, suitable for linear or nearly linear models.

• General form, suitable for nonlinear models.

• Weak form, for models with PDEs on boundaries, edges, or points, or for models using terms with mixed space and time derivatives.

Using the application modes in COMSOL Multiphysics, that can perform various types of analysis including**:**

• Stationary and time-dependent analysis.

• Linear and nonlinear analysis.

• Eigen frequency and modal analysis.

To solve the PDEs, COMSOL Multiphysics uses the proven finite element method (FEM). The software runs the finite element analysis together with adaptive meshing and error control using a variety of numerical solvers [75].

3.2.4 Work flow

To Set Up and Run a Simulation with COMSOL Multiphysics the next work flow must done as shown in **Figure (3.1)**:

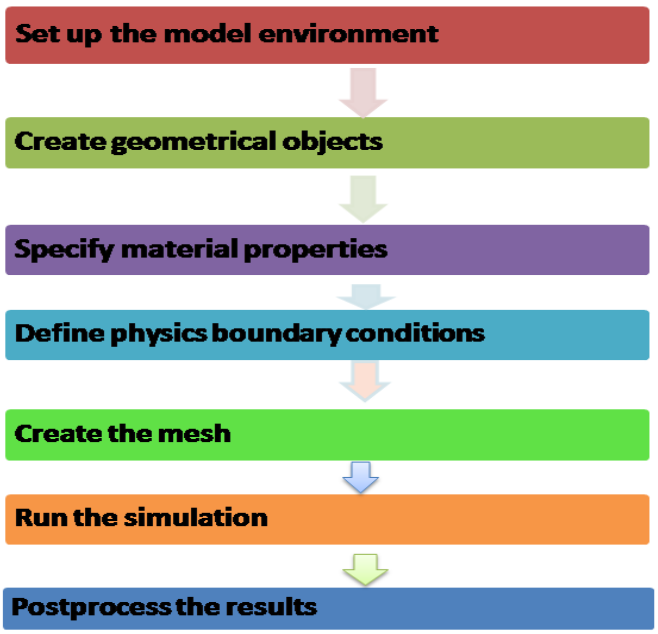

Figure 3.1: Flow chart of COMSOL Multiphysics [76]**.**

3.2.5 Application areas

There are several application-specific modules in COMSOL Multiphysics. The most common applications are [70]:

AC/DC Module, Acoustics Module, CAD Import Module, Chemical Engineering Module, Earth Science Module, Heat Transfer Module, Material Library.

3.2.6 Characteristics

The spread usage of COMSOL Multiphysics in various domains largely depends on its marked characteristics. These characteristics are [70]:

●It can be used to solve multi-physics problem.
●The user can specify their own Partial Differential Equations.
● Professional predefined modeling interfaces.
●CAD models can be made directly.
●CAD package can be added.
● Exuberance of simulation capability [71] .

One unique feature in COMSOL Multiphysics is something we refer to as extended Multiphysics, the use of coupling variables to connect PDE models in different geometries. This represents a step toward system-level modeling.
Another unique feature is the ability of COMSOL Multiphysics to mix domains of different space dimensions in the same problem. This flexibility not only simplifies modeling, it also can decrease execution time. In its base configuration, COMSOL Multiphysics offers modeling and analysis power for many application areas. For several of the key application areas we also provide optional modules. These application-specific modules use terminology and solution methods specific to the particular discipline, which simplifies creating and analyzing models [75] .

3.2.7 Application modes in Comsol multiphysics

- **Physics mode**

Use the physics modes to instantly access convenient templates for specific application areas. Here can specify physical properties for models in fields such as acoustics, diffusion, or electromagnetic.

- **Deformed mesh application modes**

These application modes provide support for applications with moving boundaries using the Moving Mesh (ALE) application mode and for parameterized geometries in 2D.

●The optimization and sensitivity analysis application modes

The Sensitivity Analysis application mode adds sensitivity analysis to any type of Multiphysics model. The Optimization application mode provides functionality for combining Multiphysics modeling with optimization (for example, topology optimization and inverse modeling).

●The PDE modes

Turn to these modes to model directly with PDEs when there cannot find a suitable physics mode. With these modes when define the problem in terms of mathematical expressions and coefficients. COMSOL Multiphysics includes three PDE modes:

• The Coefficient form allowed solving linear or almost linear problems using PDEs and coefficients that often correspond directly to various physical properties.

• The General form provides a computational framework specialized for highly nonlinear problems. Consider using a weak form for these problems, too.

• The Weak form makes it possible to model a wider class of problems, for example models with mixed time and space derivatives, or models with phenomena on boundaries, edges, or points as described with PDEs. In terms of convergence rate, these modes also set a computational framework suited for all types of nonlinear problems.

3.2.8 Selecting an application mode

● M

odeling using a single application mode

Most of the physics application modes contain stationary, Eigen value, and dynamic (time-dependent) analysis types. As already mentioned, these modes provide a modeling interface that lets performed modeling using material properties, boundary conditions, and initial conditions. Each of these modes comes with a template that automatically supplies the appropriate underlying PDE. If cannot found a physics mode that matches a given problem, try one of the PDE modes, which allowed to define a custom model in general mathematical terms. Indeed, COMSOL Multiphysics can model virtually any scientific phenomena or engineering problem that originates from the laws of science.

● Modeling Multiphysics or systems with several dependent variables

When modeling a real-world system, you often need to include the interaction between different kinds of physics. For instance, the properties of an electronic component such as an inductor vary with temperature [75].

3.3 Modeling of biosensor

3.3.1 Mathematical modelling

A mathematical model of a physical law is a description of that law in the language of mathematics. Such models make it possible to use mathematical methods to deduce results about the physical world that are not evident or have never been observed. Mathematical modelling is a technique which builds on a firm understanding of the basic terminology, notation, and methodology of mathematics. It involves the following steps. First, the problem or objective of the study must be stated in a way that reflects accurately the needs of the organization. The second step includes finding data relevant to the problem which can be applied to the model, and often includes the scaling of these measurements. This process often yields a more realistic model, the results of which are more easily comprehended. The third step in the modelling process is the development of a mathematical model that addresses the concerns of the organization. In developing the mathematical model, the primary goal is to provide a quantitative structure for analyzing a large group of possible situations. Model formulation frequently includes the selection of the appropriate mathematical functions to explain the phenomenon. In the fourth step, the data collected at the second step are applied to the mathematical model to obtain quantitative results. Step five involves the interpretation of the analysis completed in the previous step. It is very important that the results are interpreted in a clear and comprehensible way. Next, the results of the analysis are verified as to their applicability to a wide range of possibilities for the organization. The ability of a model to predict accurately is fundamental to verification. If the model is verified as useful to the organization, then it will be implemented. After implementation, use of the model may lead to additional applications for similar models, adjustments and refinements of the model. Or eventual rejection of the model if it is found inapplicable to function.Mathematical models and the modelling process serve as learning aids by emphasizing the applied aspects of mathematical analysis [15].

3.3.2 Computational modeling

The computational modelling is applied in various scientific areas, including more theoretical ones (alternating direction method for solving Poisson [77]. and parabolic

equations [[28]- [29]] as well as for solving applied problems when modelling blood glucose dynamics , anisotropic media , moisture diffusion in wood [30], piezoelectric and ultrasound actuators , protein spot detection and others. Computational modelling is the only way to solve the problems presented by the mathematical models of the biosensors since the analytical solutions exist only at extreme set of parameter values [10].

3.4 Modeling of our biosensor under comsol multyphysics 5.4

3.4.1 Model definition

Many enzyme catalyzed reactions are exothermic, generating very small amounts of heat. Temperature changes are generally determined by thermistors at the inlet and outlet of the microchannels containing immobilized enzymes in a fixed bed in a constant temperature environment. In this work, we are interested in studying and simulating the mechanism of operation of a biosensor based on enzymatic reactions. Indeed, enzymatic reactions are generally exothermic. We want through our simulations to predict the amount of heat released (The figure below shows the principle working of our Biosensor)

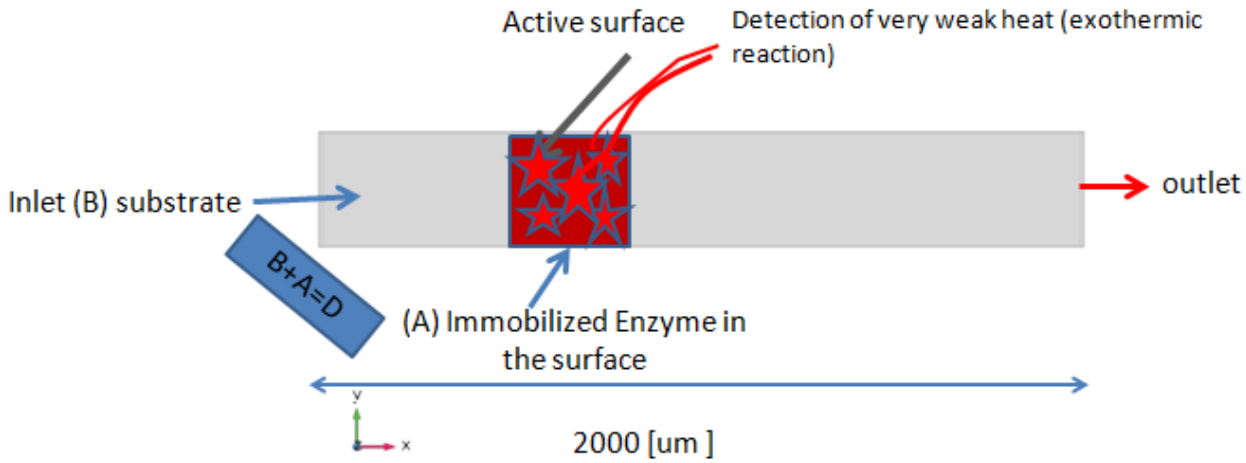

Figure 3.2: Principle working of our Biosensor .

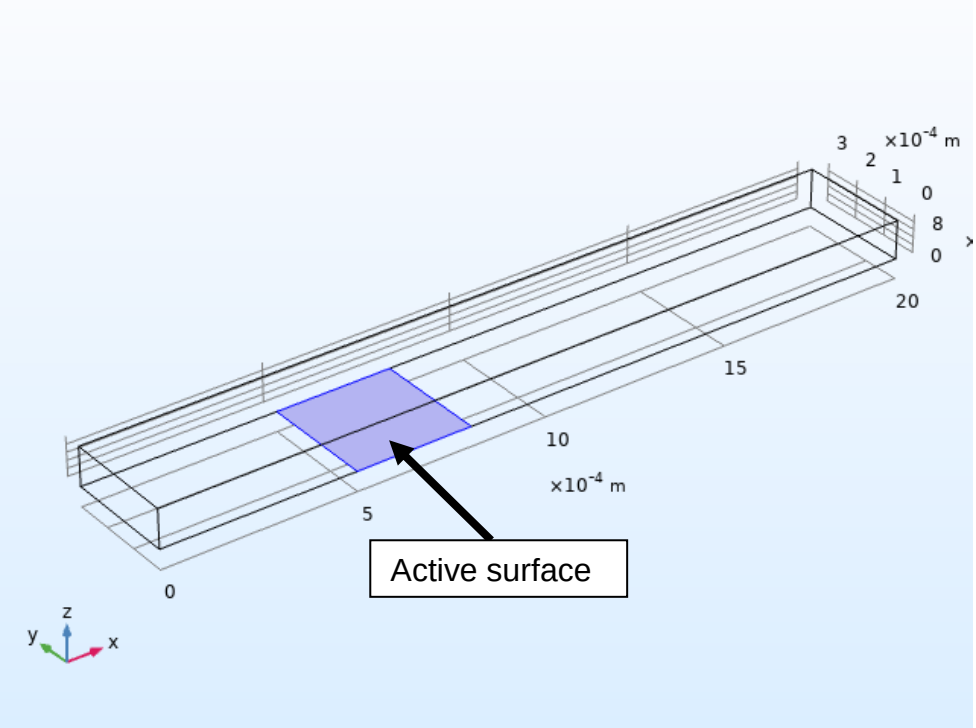

Figure 3.3: Simulation of our enzymatic biosensor with Comsol 5.4.

3.4.2 Model parameters

For the simulation under comsol we have chosen Parameters in the Table below:

Name	Value	Description
k_ads	0.01 m/s	Forward rate constant
k_des	0.5 mol/(m²·s)	Backward rate constant
D	$2.4.10^{10}$m²/s	Gas diffusivity
kf	$2.\ 10^{-7}$mol/(m²·s)	Forward rate constant
kr	$4.\ 10^{-9}$ mol/(m²·s)	Reverse rate constant
T0	293.15 K	Initial temperature
Af1	0.01 m²/(s·mol)	Frequency factor for enzymatic reaction
Ef1	40000 J/mol	Activation energy
H1	-53000 J/mol	Enthalpy of enzymatic reaction
hx	100 W/(m²·K)	Heat transfer coefficient of pdms
Mn_A	56.5 kg/mol	Molar mass, A of Cholesterol oxidase enzyme
Mn_B	0.386 kg/mol	Molar mass, B of Cholesterol substrate
Mn_D	56.886 kg/mol	Molar mass,D product of reaction
c_A0mass	$7.64.\ 10^{-5}$kg/m²	mass surface concentration enzyme A
cB1mass	0.02 kg/m³	mass substrate concentration
cB10	0.051813 mol/m³	Concentration B, inlet 1

c_A0	1.3522. 10^{-6} mol/m²	Surface concentration at surface sensing
ll	2000 µm	microchannel length
ww	300 µm	Microchannel width
hh	100 µm	Microchannel height
Q_in	0.180 m³/s	Debit fluid entree
U1	1. 10^{-4}m/s	Velocity, inlet 1

Table 3.1: Parameter of Biosensor .

3.4.3 Geometry of the model

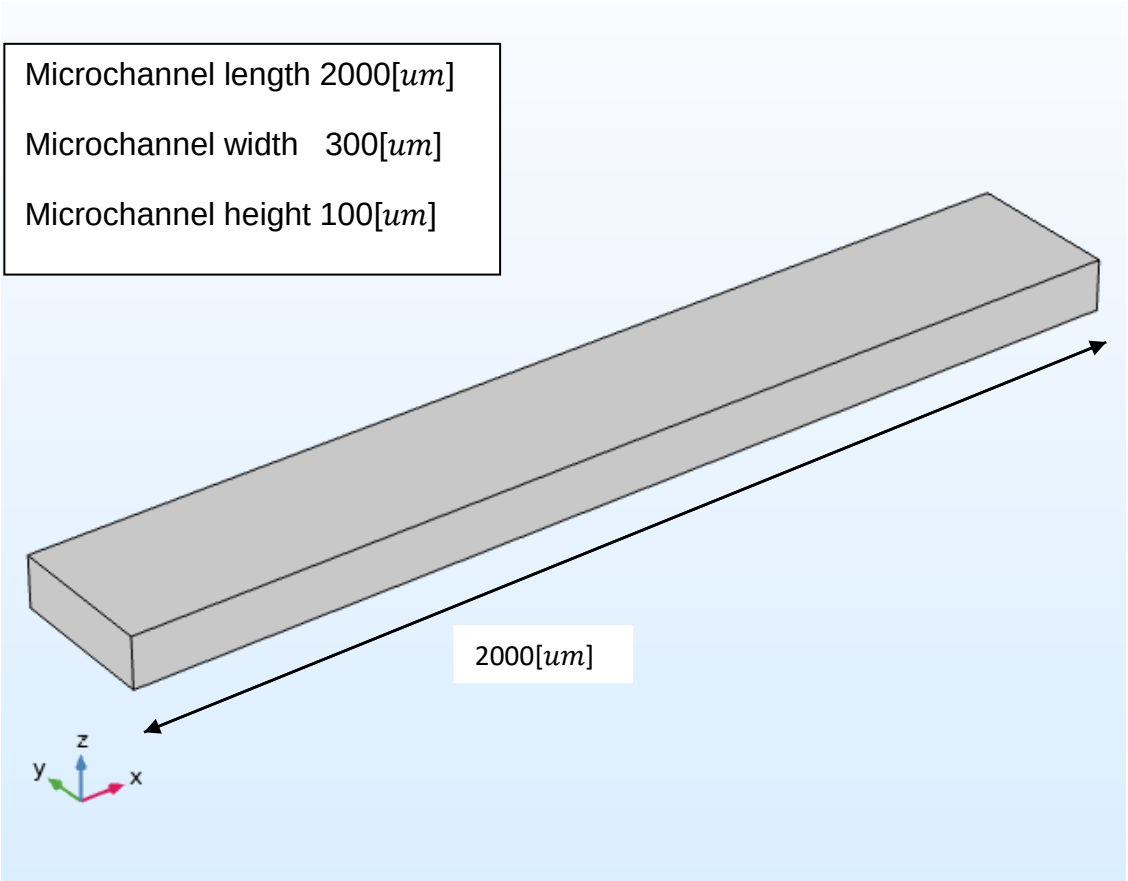

Figure 3.4: Geometry of the model using comsol 5.4 .

3.4.4 The physics entering in our model

3.4.4.1 Laminar flow (spf)

It's used to calculate the velocity field $U^{\rightarrow}$ (u, v, w) and pressure (P) for the flow of a fluid in the laminar flow regime.In this physics we have chosen an Inompressible flow

The equations solved by the laminar flow interface are the Navier-Stokes equations:

$$\rho(\mathbf{u}.\nabla)\mathrm{u} = \nabla \cdot [-PI + \mathbf{K}] + \boldsymbol{F}$$

$$\rho\nabla \cdot (\mathbf{u}) = 0$$

$$\mathbf{K} = (\nabla\mathbf{u} \cdot (\nabla\mathbf{u})^{T})$$

The laminar flow interface is used for stationary analysis with:

U1= 1. 10^{-4}m/s at the Inlet 1 and P0= 0 Pa at the outlet

The figure below show the Laminar flow physics under comsol 54

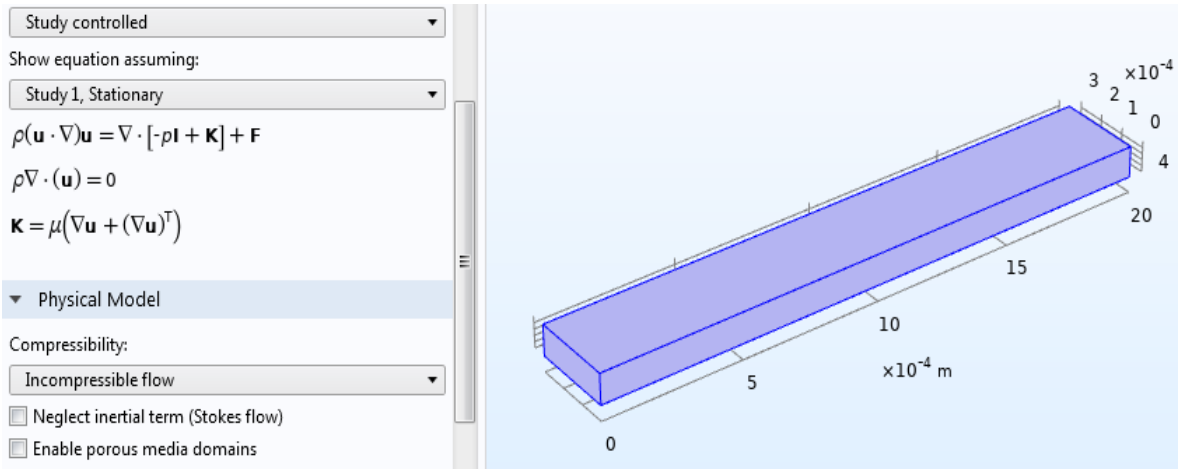

Figure 3.5: Laminar flow (spf) .

3.4.4.2 Transport of diluted species (chds)

Transport of diluted species Physics is used to calculate the concentration range of diluted solute (cB) in a solvent ($H2O$) Driving forces for transport can be spread by Fick's law

$\nabla . \mathbf{J}_i + \boldsymbol{u}. \nabla C_i = R_i$ $\qquad\qquad$ $\mathbf{J}_i = -D_i \nabla C_i$

D: ($m^2. s^{-1}$) :Diffusion coefficient $\qquad$ $\boldsymbol{u}$: (m/s) : Velocity field

C_i :($mol. m^{-3}$) : Molar concentration of species

$\mathbf{J}_i$: ($mol. m^{-2}. s^{-1}$) : Molar flux

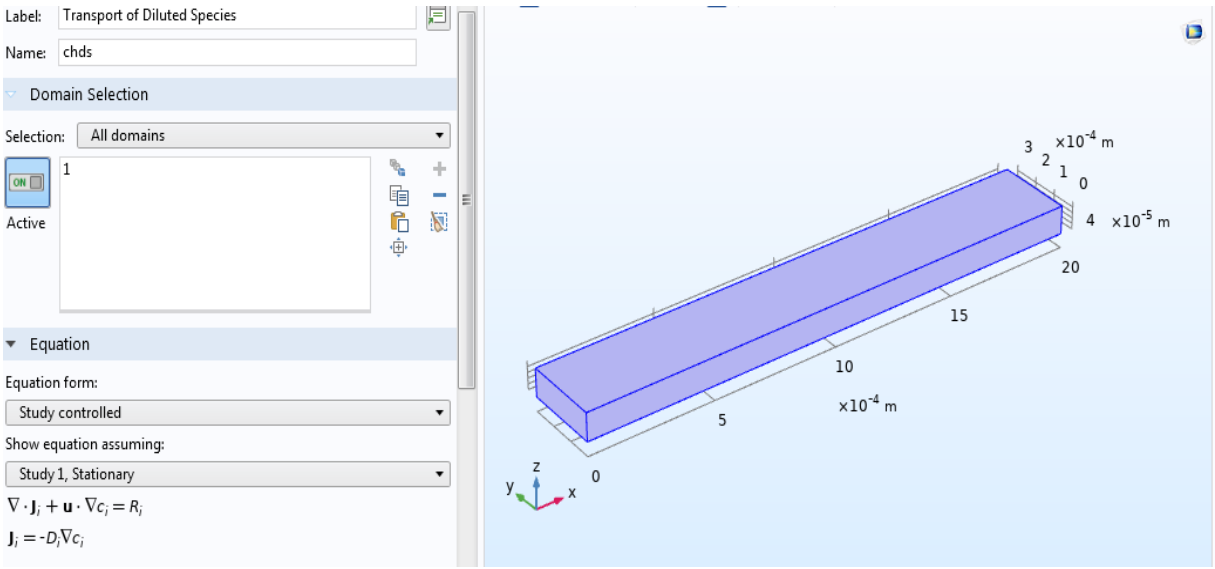

Figure 3.6: Transport of diluted species (chds) .

3.4.4.3 Surface reaction (chsr)

The analysis molecules (B) can adsorb and desorb from the surface sites (A) on the planar surfaces according to:

$$B(ads) + A(ads) \underset{K_{des}}{\overset{K_{ads}}{\rightleftharpoons}} D(ads)$$

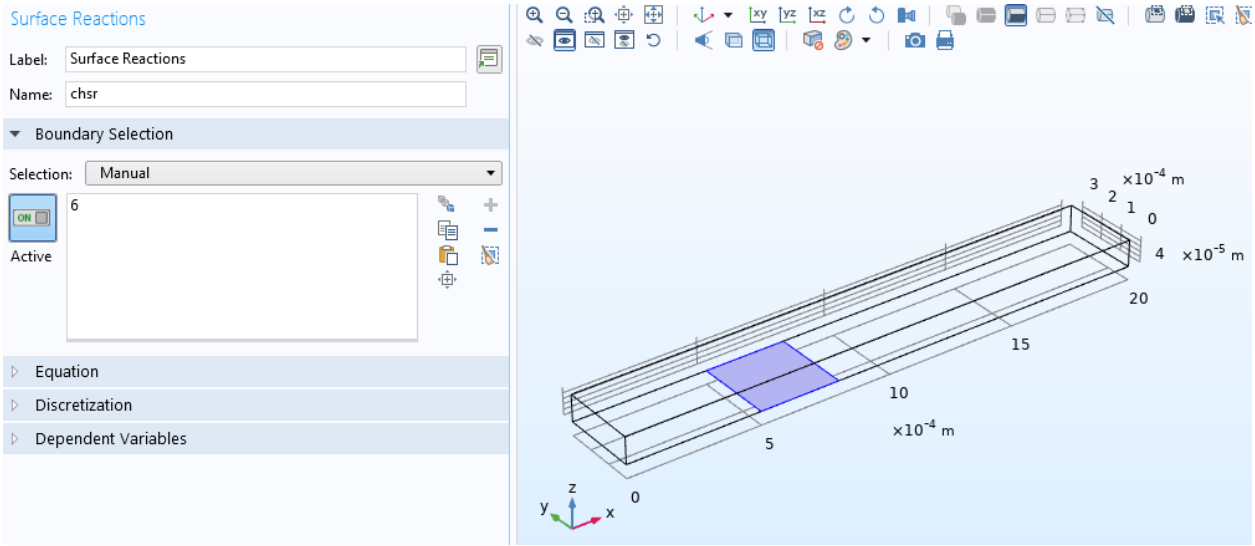

Figure 3.7: Surface reaction (chsr) .

3.4.4.4 Chemistry (chem)

This physical interface can be used to create reaction kinetics and possibly calculate transport and thermodynamic properties for a direct use:

Model Input :

- The temperature is taken from the heat transfer physics(ht (we gonna talk about it in the next section)).
- Pressure 1[atm]
- Concentration:

Specie B(ads)	Specie A(ads]	SpecieD(ads)
cB_surf	cA_surf	cD_surf

Table3.2: Concentration entering in Chemistry (chem.) Physics .

- Reaction formula :

(Irreversible reaction) B(ads)+A(ads)=>D(ads)

- Reaction rate :

$$r_j = K_f^j \prod_{i\varepsilon reactt} c_i^{-vij}$$

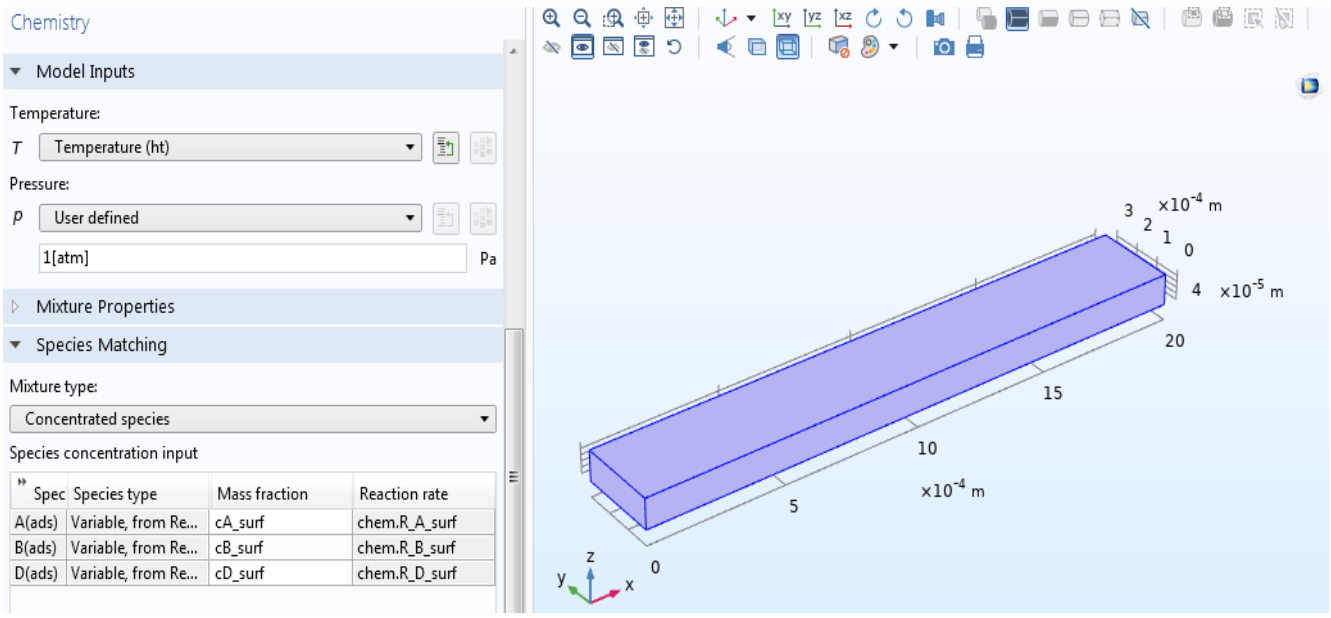

Figure 3.8a: Chemistry (chem.) Input .

- In this physics we use also The Arrhenius expressions :

$$K^f = A^f(T|T_{ref})^{n^f} exp(-E^f|R_jT)$$

$$T_{ref} = 1K$$

With

$$A^f = Af1 \quad E^f = Ef1 \quad n^f = 0\text{(Look parameter Table)}$$

- Enthalpy of reaction : H=H1= -53000 J/mol
- Reaction Heat : $Q_j = -r_jH_j$

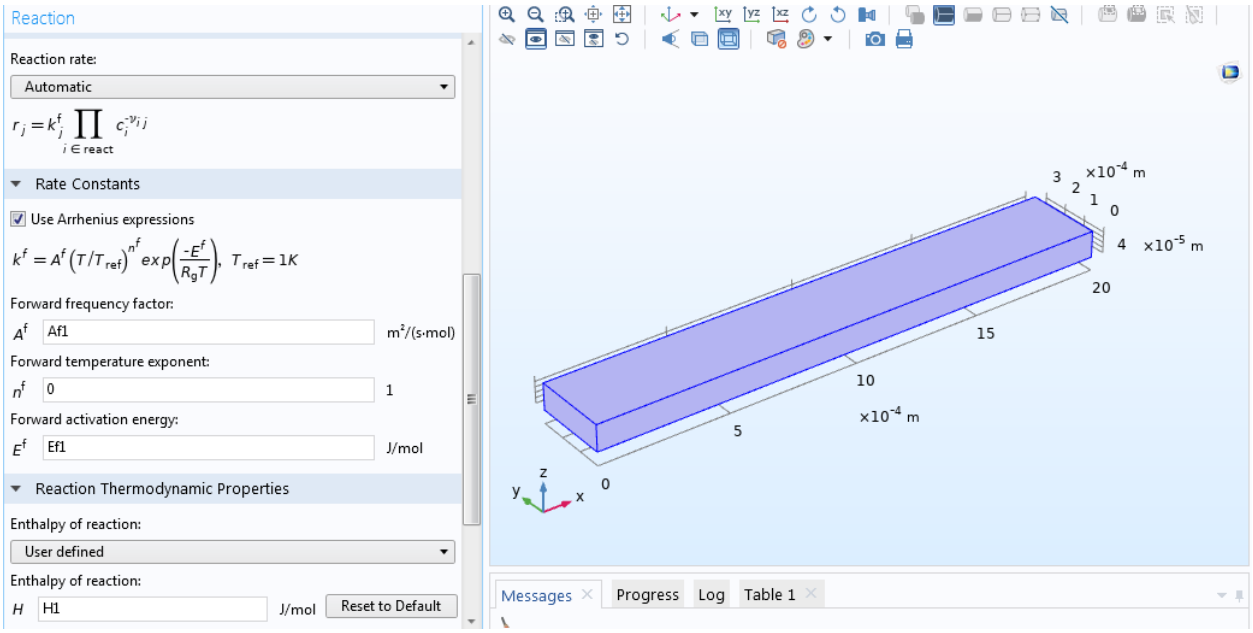

Figure 3.8b: Chemistry (chem) Input .

3.4.4.5 Heat transfer in fluids (ht)

The physics of heat transfer in fluids is used to model heat transfer by conduction, convection and radiation. This physics uses the following version of the heat equation to model the heat transfer in the fluid:

$$\rho C_P \mathbf{u}.\nabla T + \nabla.q = Q + Q_p + Q_{vd}$$

$$\boldsymbol{q} = -K\nabla T$$

ρ : (Kg/m^3) fluid density K :(W/(m.K)) thermal conductivity of fluids

C_P : (J/(Kg.K)) the heat capacity of the fluid at constant pressure

u : (m/s) fluid velocity field Q : (W/m^3) heat source

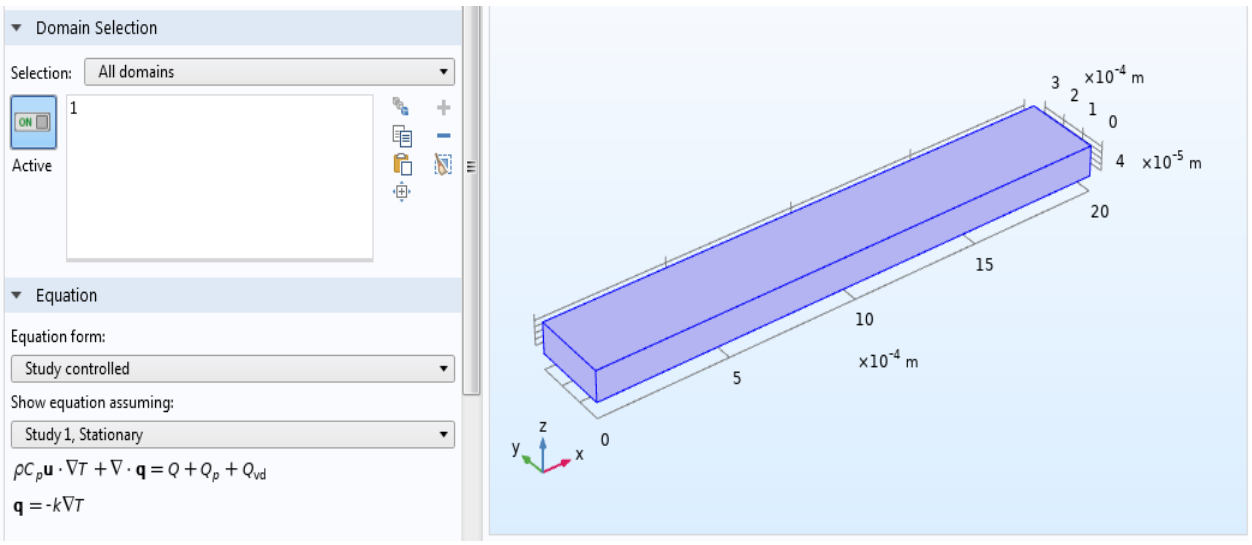

Figure 3.9a: Heat transfer in fluids (ht) .

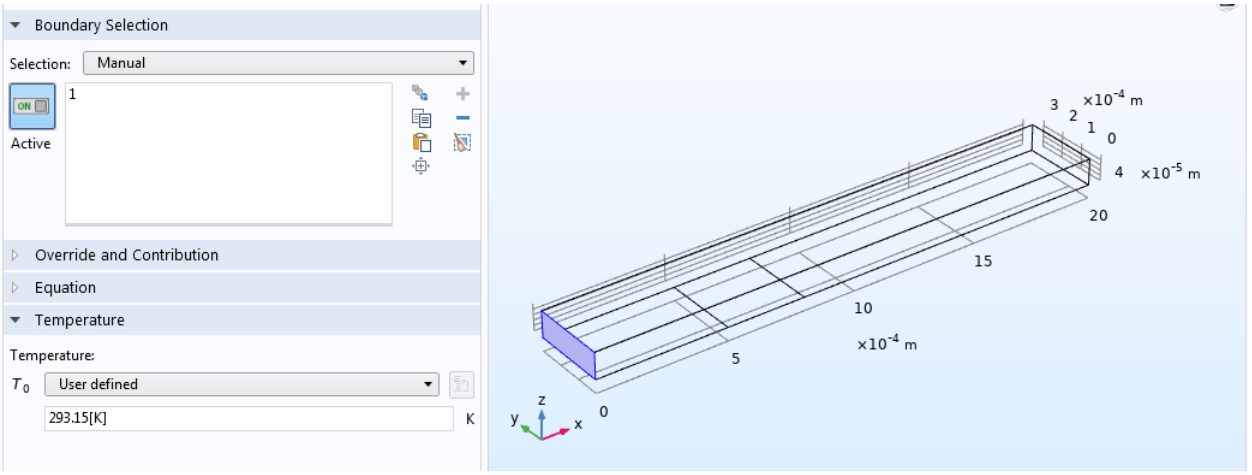

Figure 3.9b: Temperature at Inlet T0=293.15[K] .

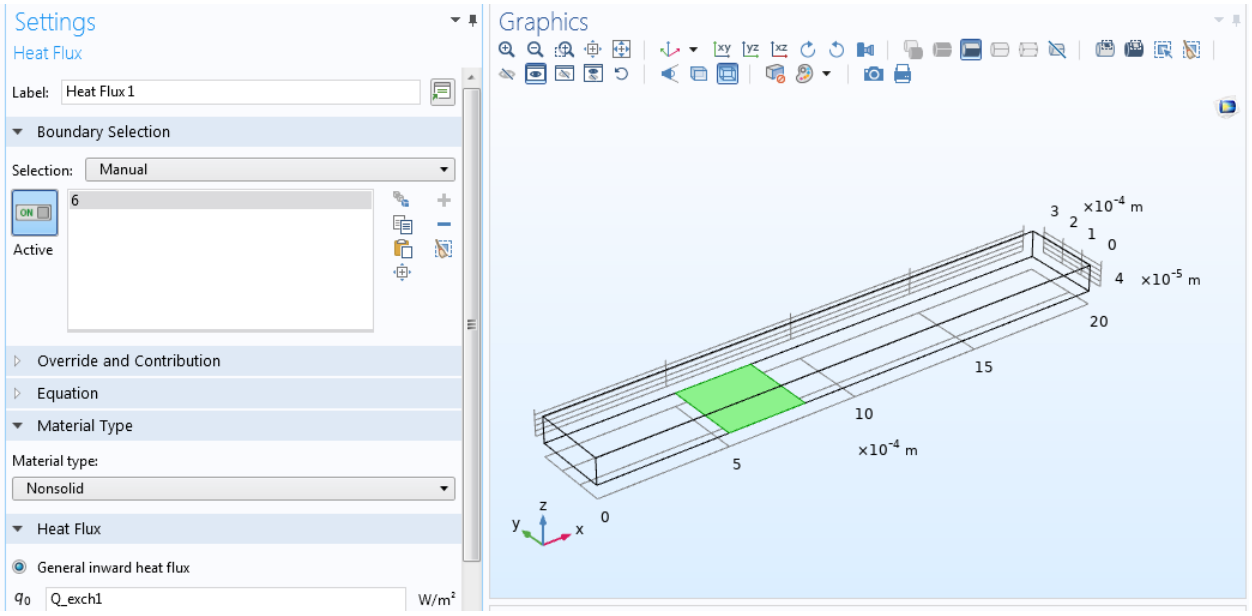

Figure 3.9c: Heat flux boundary condition .

Q_exch1= (T0-T)*hx [W/m^2]

3.4.5 Multiphysics

An empty **Multiphysics** node is added automatically when two (or more) physics interfaces are set up in a model and when there is the possibility to couple the physics interfaces. The **Multiphysics** node ( ) contains, or has available, any coupled physics features that are likely to be used for a particular set of physics interfaces added to the Model Builder.

- **Flow coupling**

The **Flow Coupling** multiphysics coupling ( ) defines **u** and *p* variables in order to set the model inputs in the Heat Transfer interface (or when applicable, a chemical species transport interface). In addition it provides all the fluids quantities that may be needed by the Heat Transfer interface

- **Temperature coupling**

Use this multiphysics coupling to add the temperature as the default model input for a standalone physics interface. The **Temperature Coupling** feature is generic and specifies a Heat Transfer interface as **Source** and a second interface as **Destination**. When **Temperature Coupling** feature is used, the temperature from the **Source** is used to evaluate material properties in any feature from the **Destination** interface. The coupling can be added wherever the Heat Transfer interface is active. The **Source** interface can be any interface defining a temperature, which includes all versions of heat transfer and multiphysics, except the pure radiation interfaces. The **Destination** interface can be any interface providing multiphysics feature in the **Multiphysics** node.

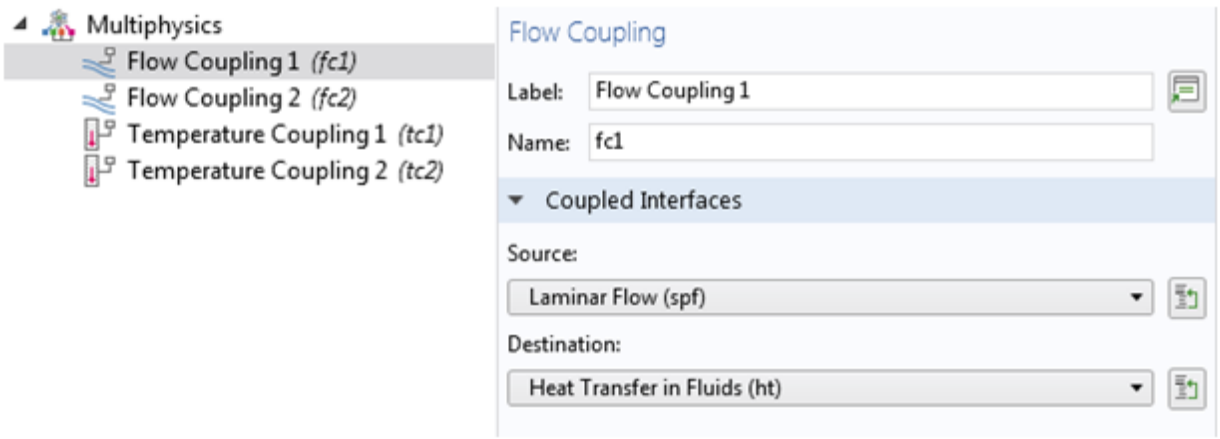

Figure 3.10a: Multiphysics node and coupled interfaces for flow coupling 1 (fc1)

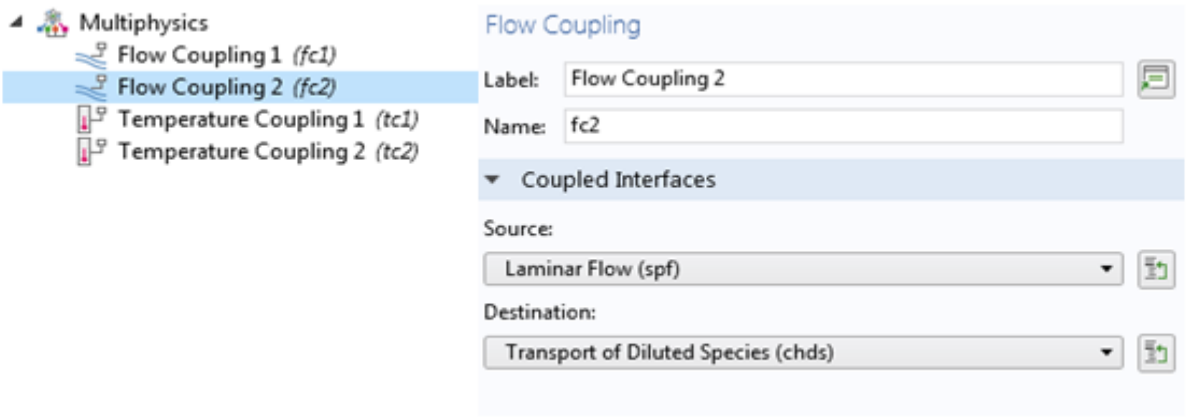

Figure 3.10b: Multiphysics node and coupled interfaces for flow coupling 2 (fc2)

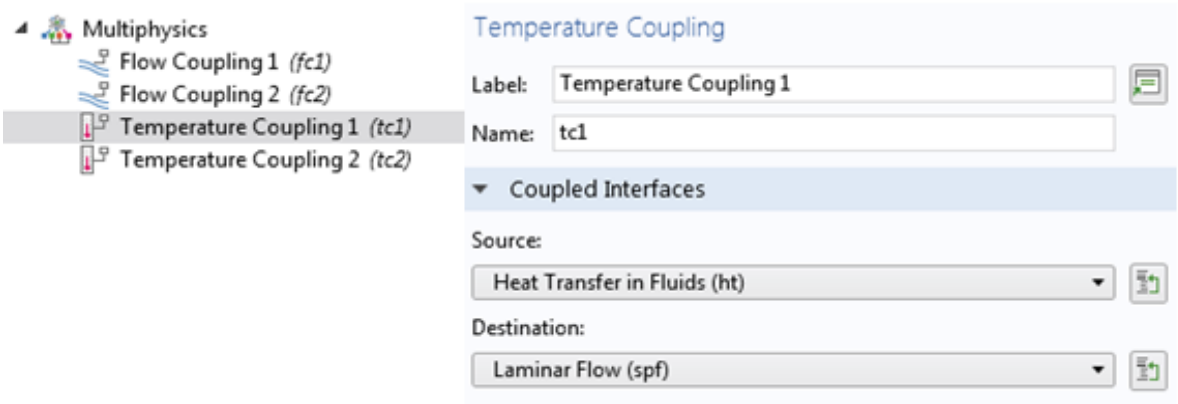

Figure 3.10c: Multiphysics node and coupled interfaces for temperature coupling 1 (tc1).

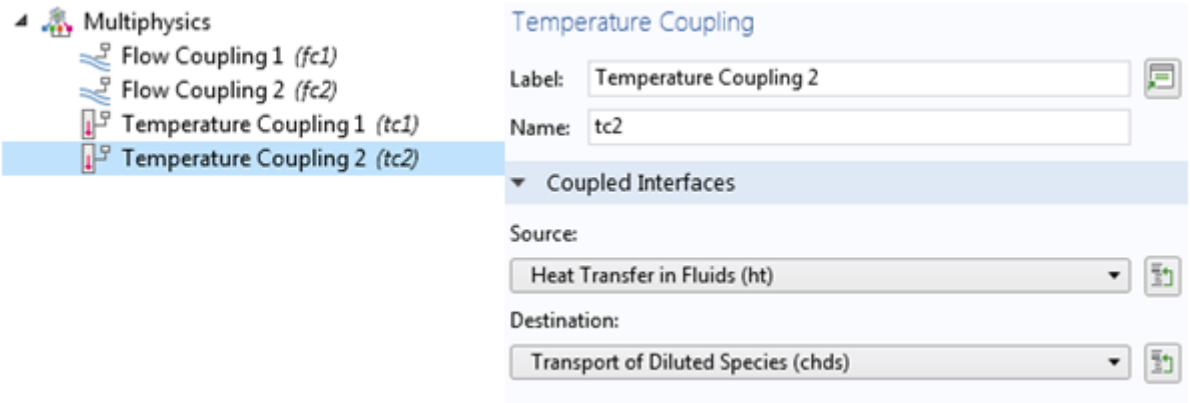

Figure 3.10d: Multiphysics node and coupled interfaces for temperature coupling 2 (tc2) .

3.4.6 The Meshing

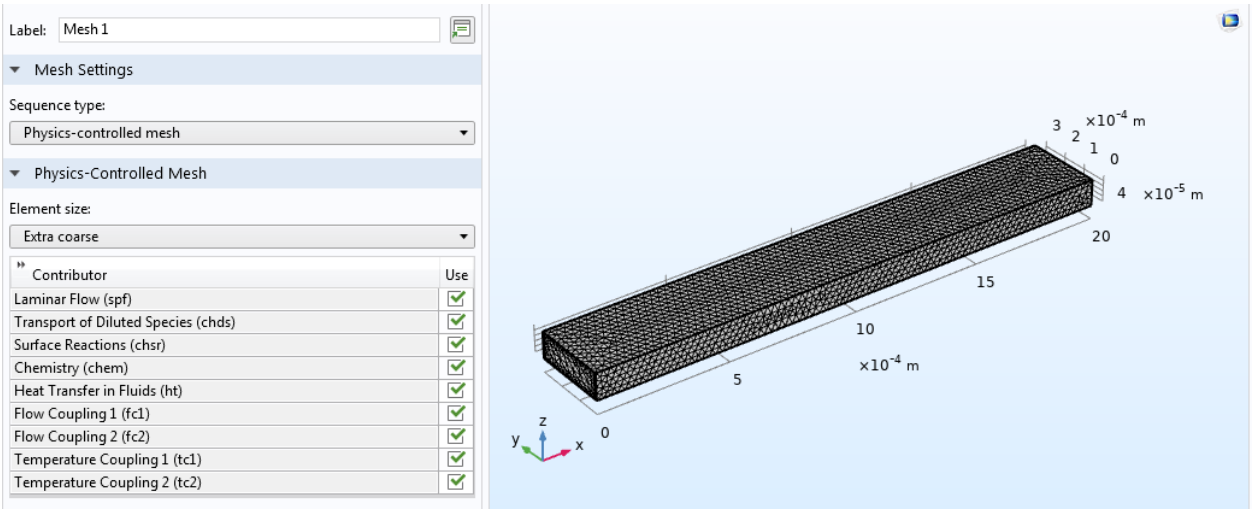

Figure 3.11: The Meshing of the Model .

3.4.7 Study

- Step 1: stationary : In this step we want that the programme (comsol(5.4)) simulate only the Laminar flow (spf) physics with all the coupled interfaces.

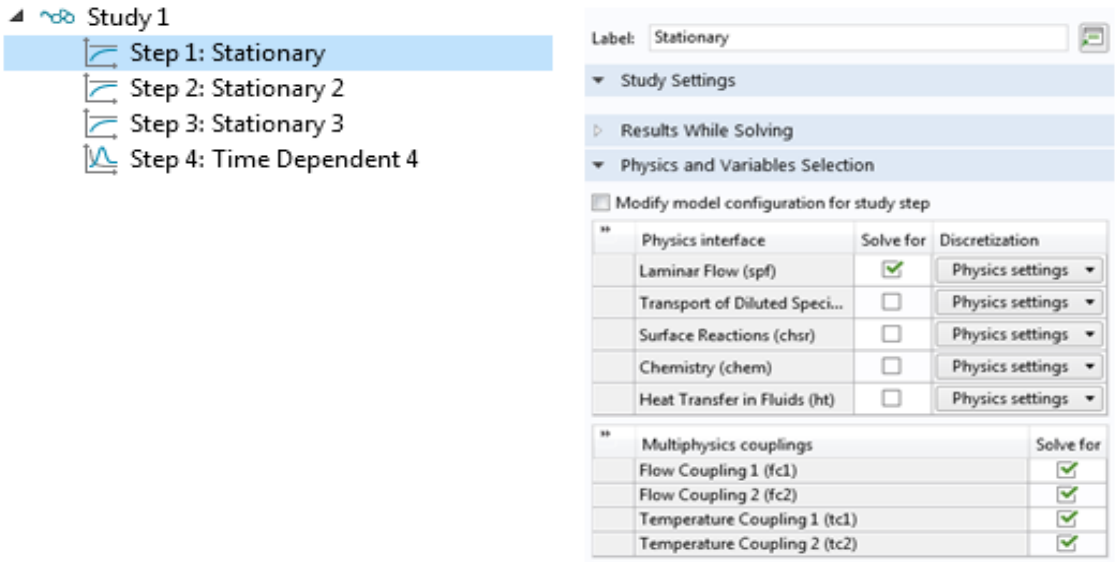

Figure 3.12a: Step 1 stationary .

- Step 2: Stationary 2: In this step we want that the programme (comsol(5.4)) simulate all the other four physics except the heat transfer in fluids (ht) with all the coupled interfaces.

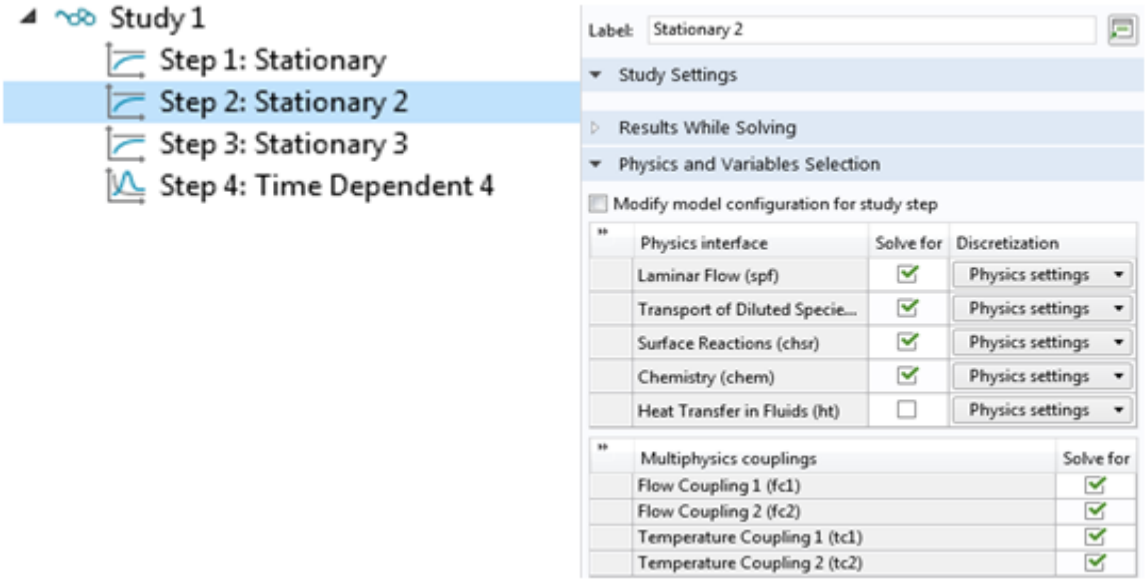

Figure 3.12b: Step 2 Stationary .

- Step 3: Stationary 3: In this step we want that the programme (comsol (5.4)) simulate all the five physics with all the coupled interfaces.
- Step 4: Time dependent: In this step we want that the programme (comsol(5.4)) simulate all the five physics with all the coupled interfaces. We decide also that the range of time start from 0s to 100s with a step of 1sec .

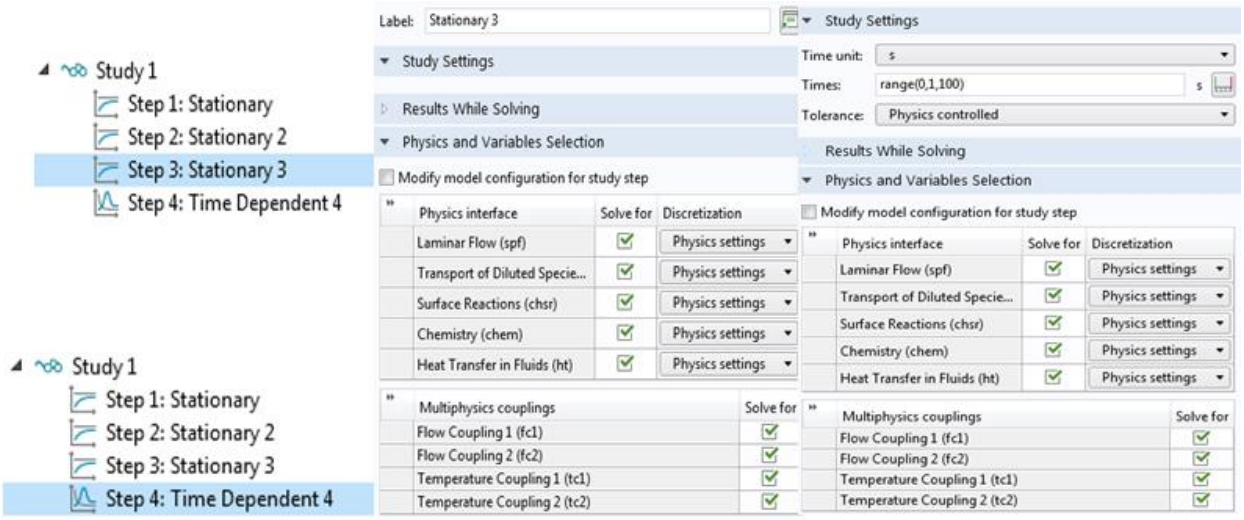

Figure 3.12c: Step 3 (stationary) + step4 (time dependent) .

3.5 Conclusion

In this chapter our team had tried to modelate our biosensor which contains several physics which makes the task a little bit difficult because when we treat several physics (multiphysics) the simulation under comsol becomes too much difficult, so after several attempts we managed to make this simulation to be able to exploit the results.

CHAPTER 4

RESULTS AND DISCUSSION

(Note): We present the results of the simulations by observing the velocity, the temperature change along the microchannel, as well as the evolution of the concentrations of the species during the flow.

4.1 Velocity (spf)

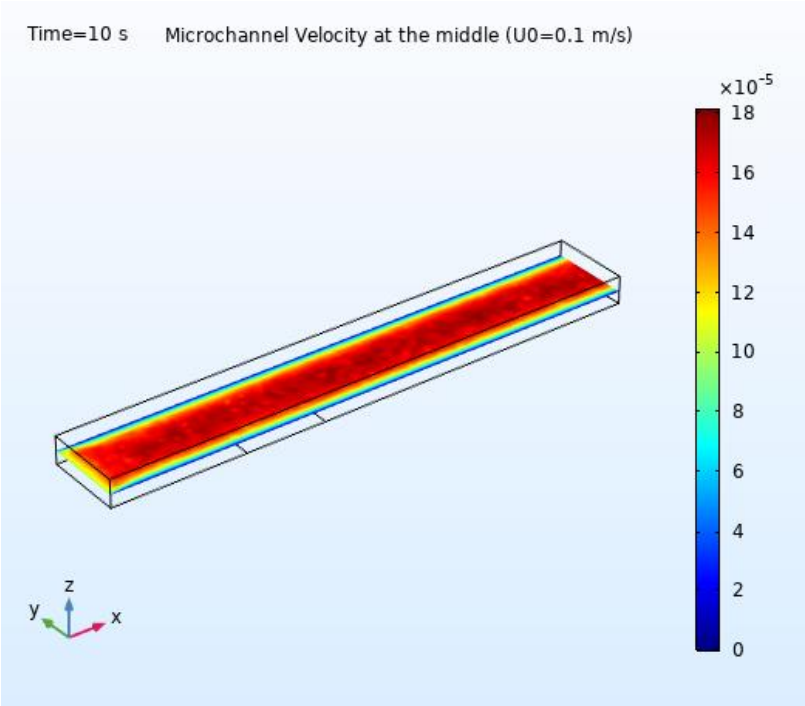

Figure 4.1: Microchannel velocity (xy Plane) .

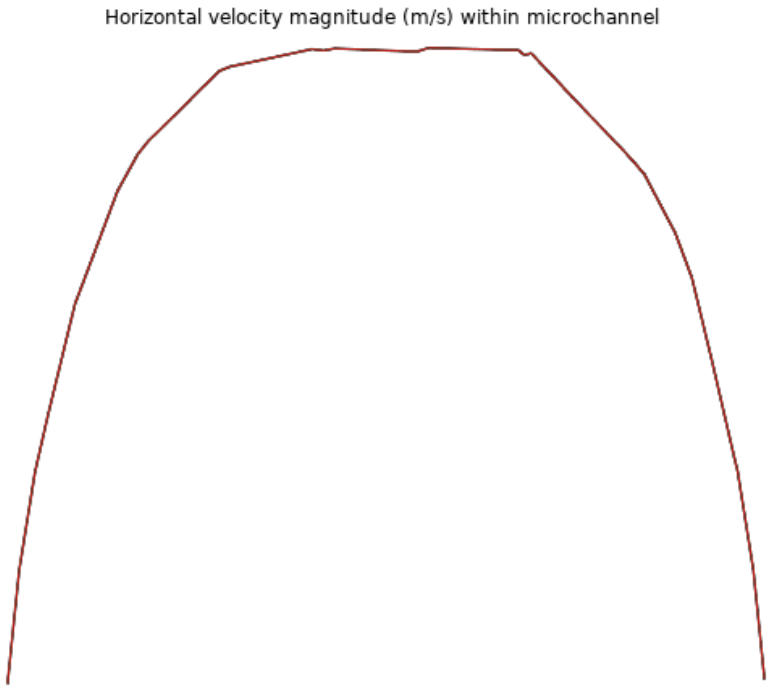

Figure 4.2: Horizontal velocity magnitude (m/s) within microchannel .

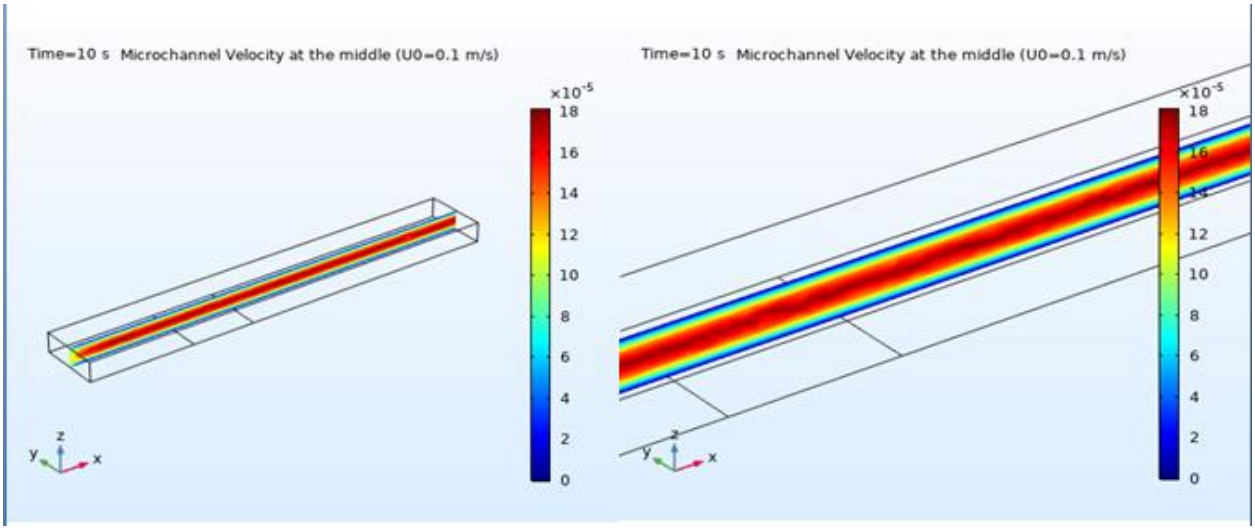

Figure 4.3a: Microchannel velocity at the middle (ZX plane) .

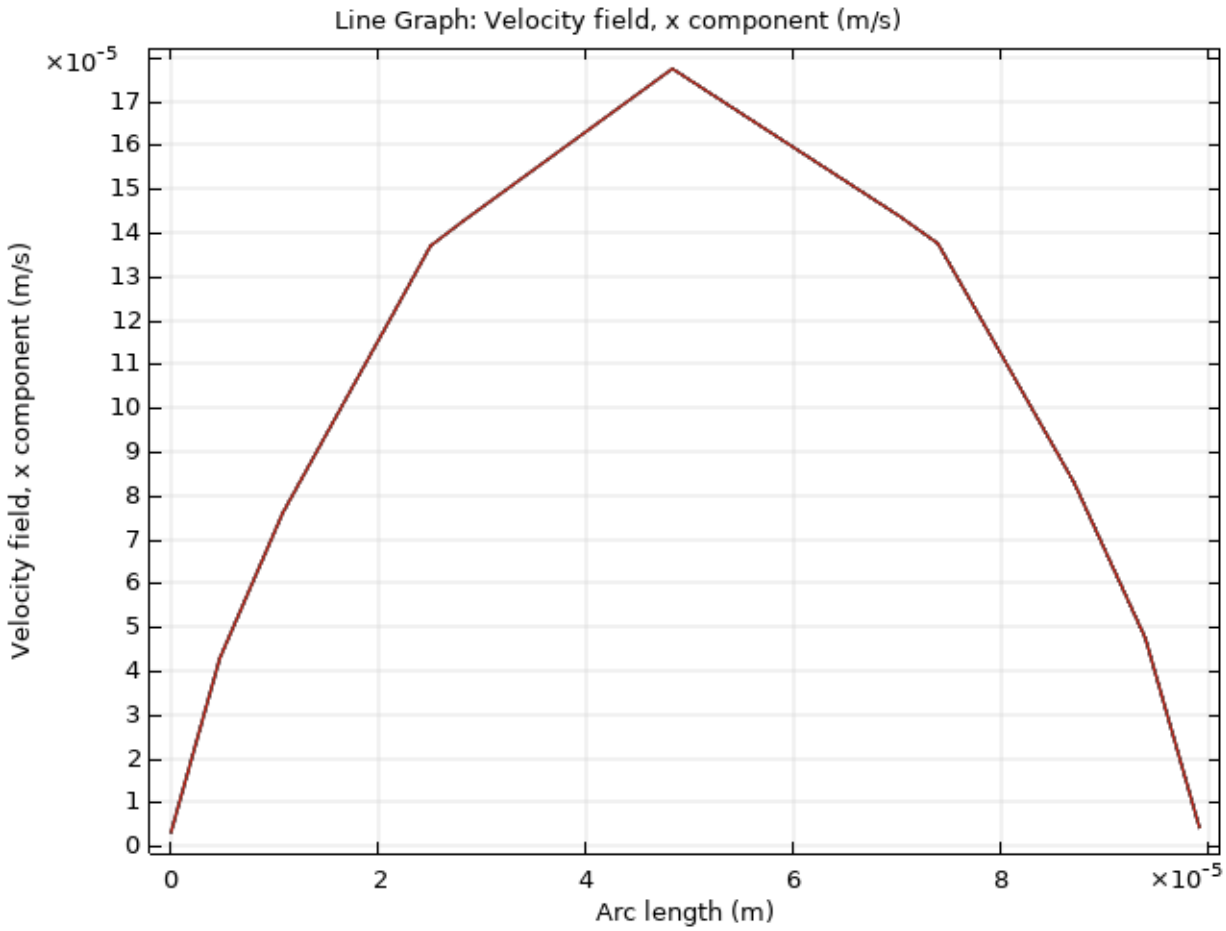

Figure 4.3b: Vertical velocity magnitude (m/s) within microchannel .

4.1.1 Discussion

The results show that the velocity has a parapolic shape (according to the graphs) and it has a maximum value in the middle of the microchannel (However if we take a cut line horizontally or vertically) than other ways.

4.2 Species concentration

4.2.1 Concentration of the substrate c_B (Cholesterol)

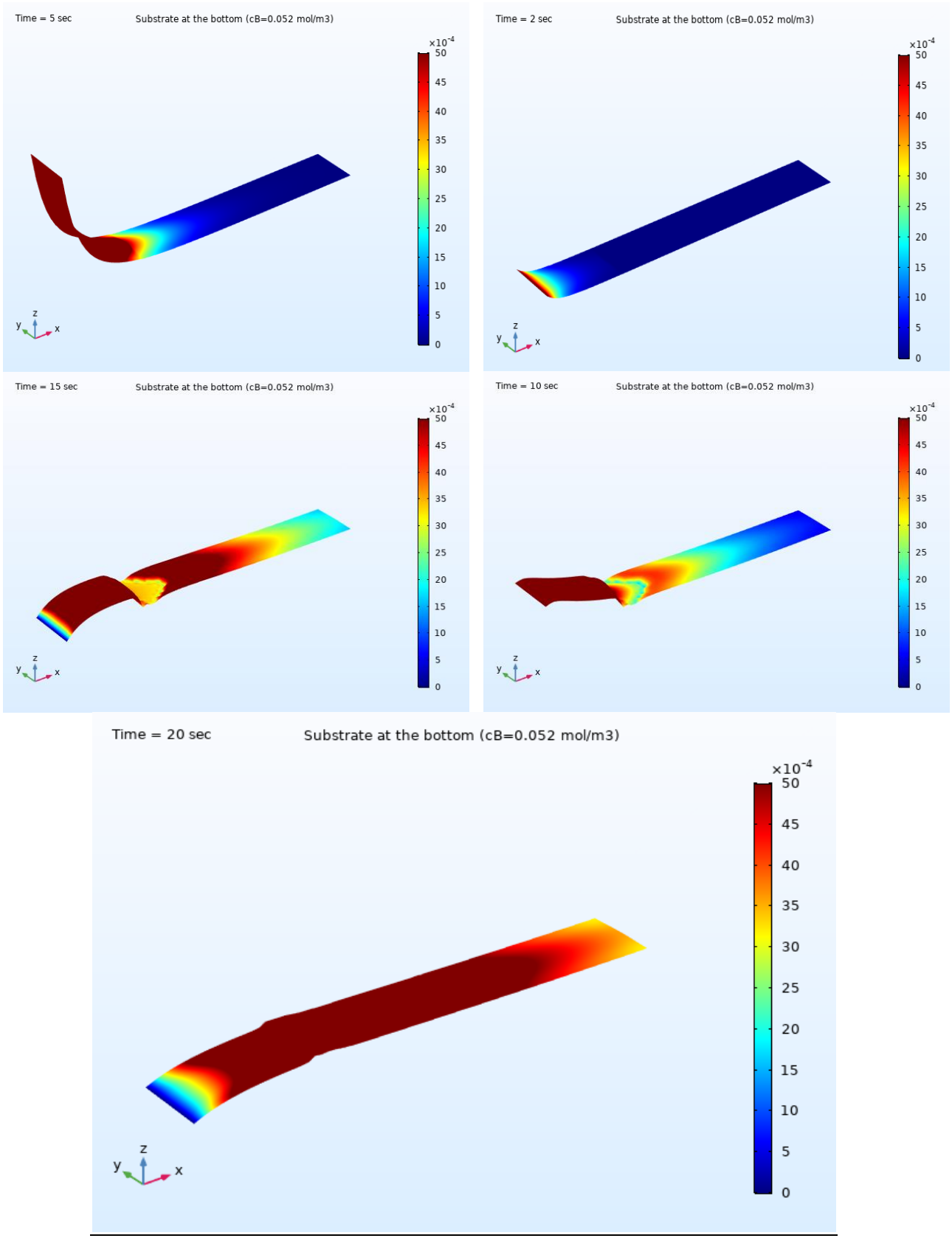

Figure 4.4: Substrate concentration c_B (Cholesterol) at the bottom of the microchannel at different time (2, 5,10,15,20 s) .

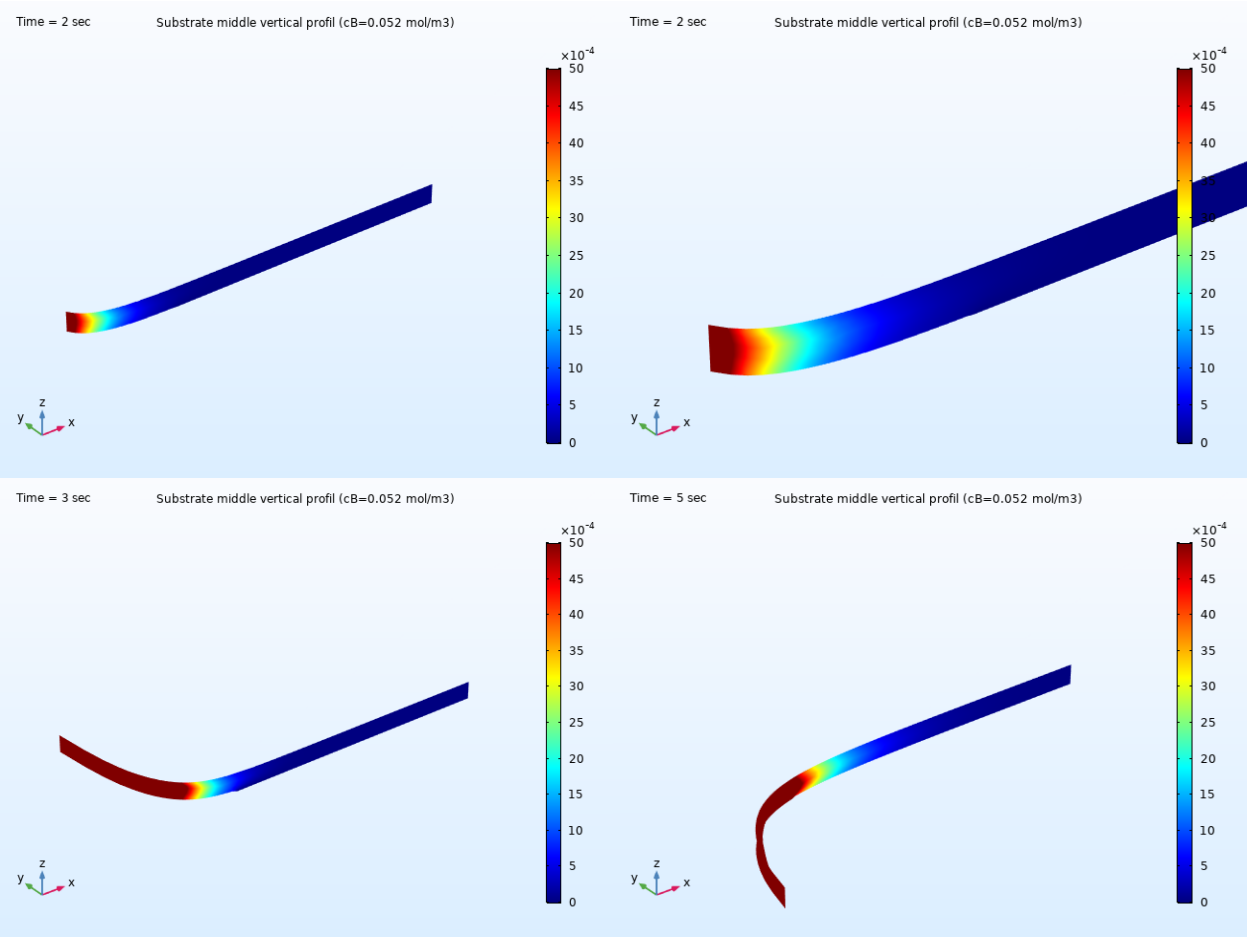

Figure 4.5: Substrate concentration c_B (Cholesterol) at the middle of the microchannel at different time (2, 3, 5 s).

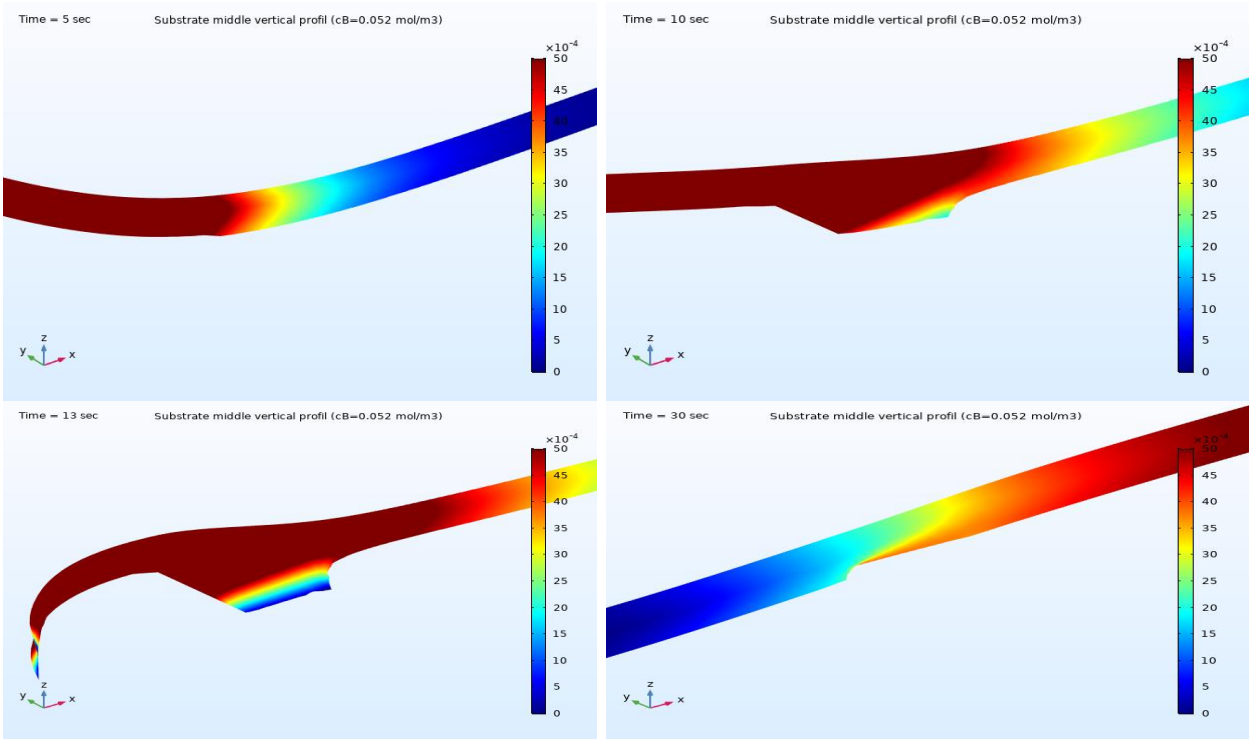

Figure 4.6: Substrate concentration cB (Cholesterol) at the middle of the microchannel at different time (10, 13,30 s).

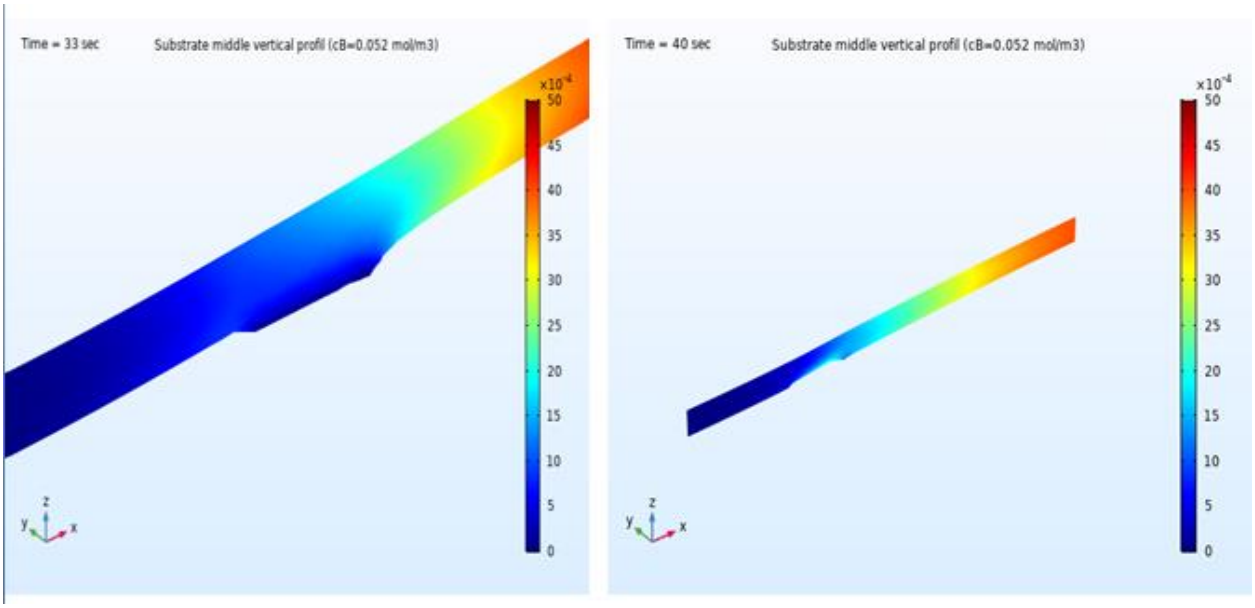

Figure 4.7: Substrate concentration cB (Cholesterol) at the middle of the microchannel at different time (33, 40 s).

4.2.1.1 Discussion

After observing the results we can say that the substrate concentration has a higher value in the beginning of the reaction than it starts to decrease with time so like we see at 40 sec the substrate concentration c_B has a low value in the middle of the microchannel.

4.3 Surface coverage of the product of the enzymatic reaction c_{D_surf}

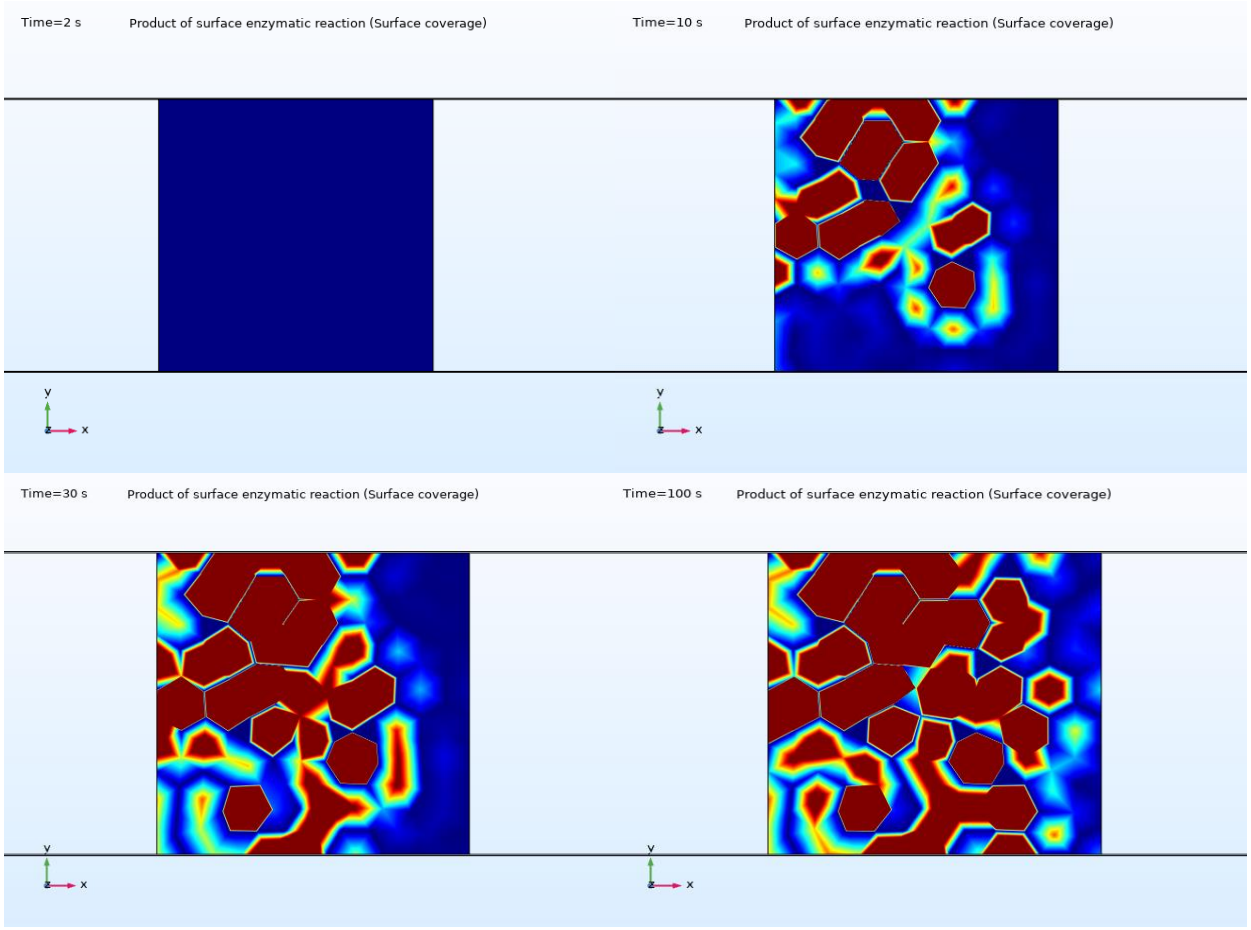

Figure 4.8: 2D surface reaction coverage of the product of enzymatic reaction c_{D_surf}.

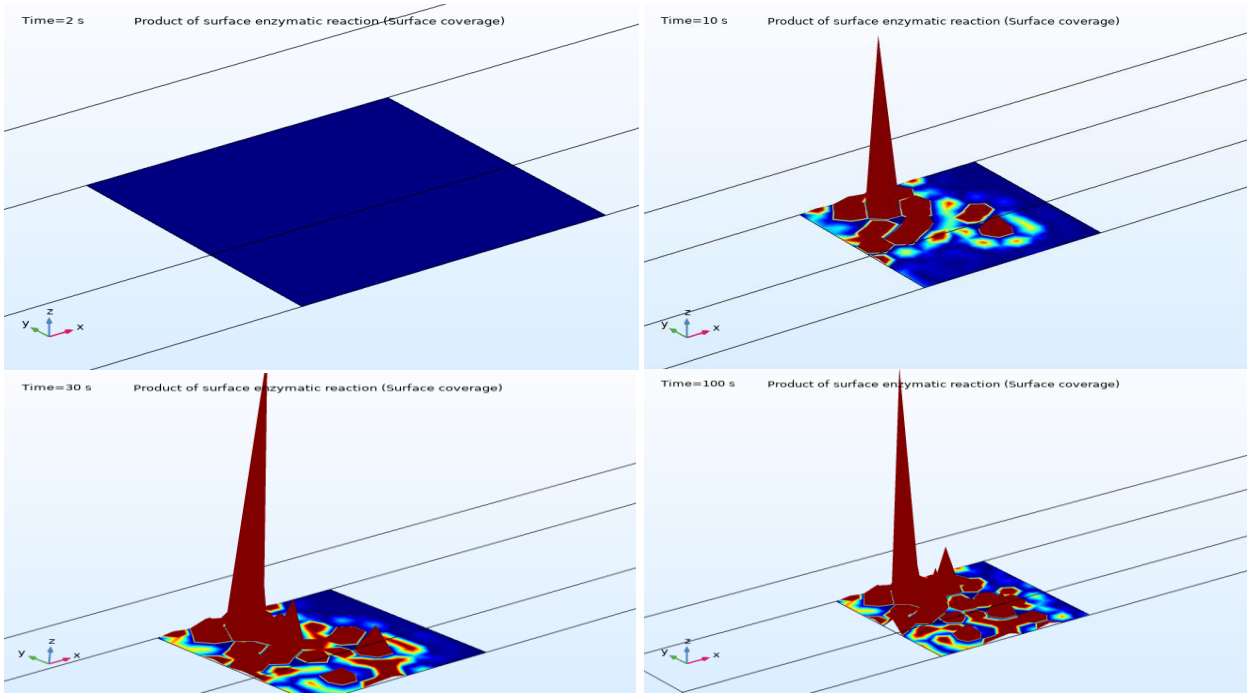

Figure 4.9: 3D surface reaction coverage of the product of enzymatic reaction c_{D_surf}.

4.3.1 Discussion

Surface coverage is the number of adsorbed molecules on a surface divided by the number of molecules in a filled monolayer on that surface. According to the results we observe that the concentration c_{D_surf} of the product of the enzymatic reaction increase quickly with time in the microchannel.

4.4 Temperature

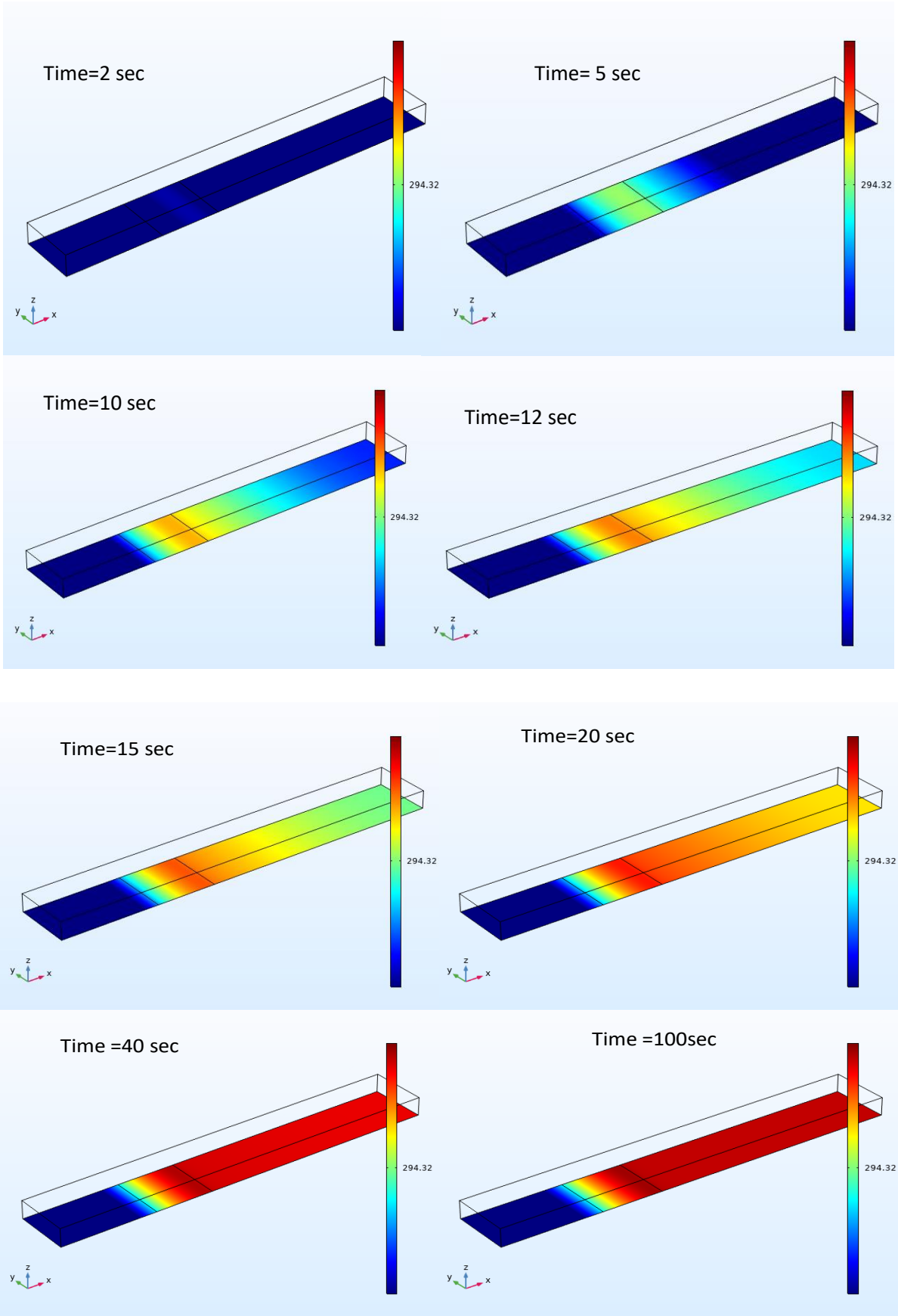

Figure 4.10: (3D) Temperature profile at the bottom of the microchannel.

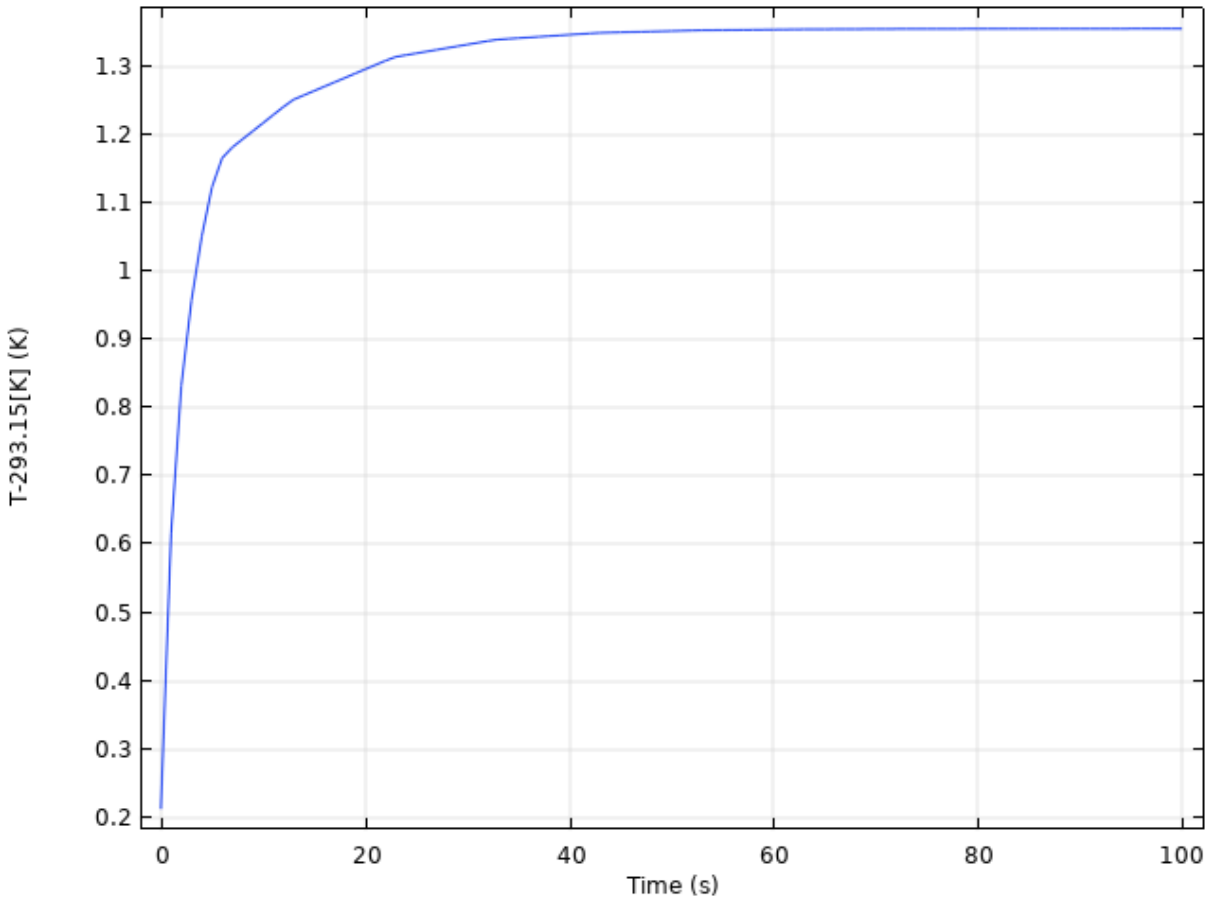

Figure 4.11: Average temperature evolution.

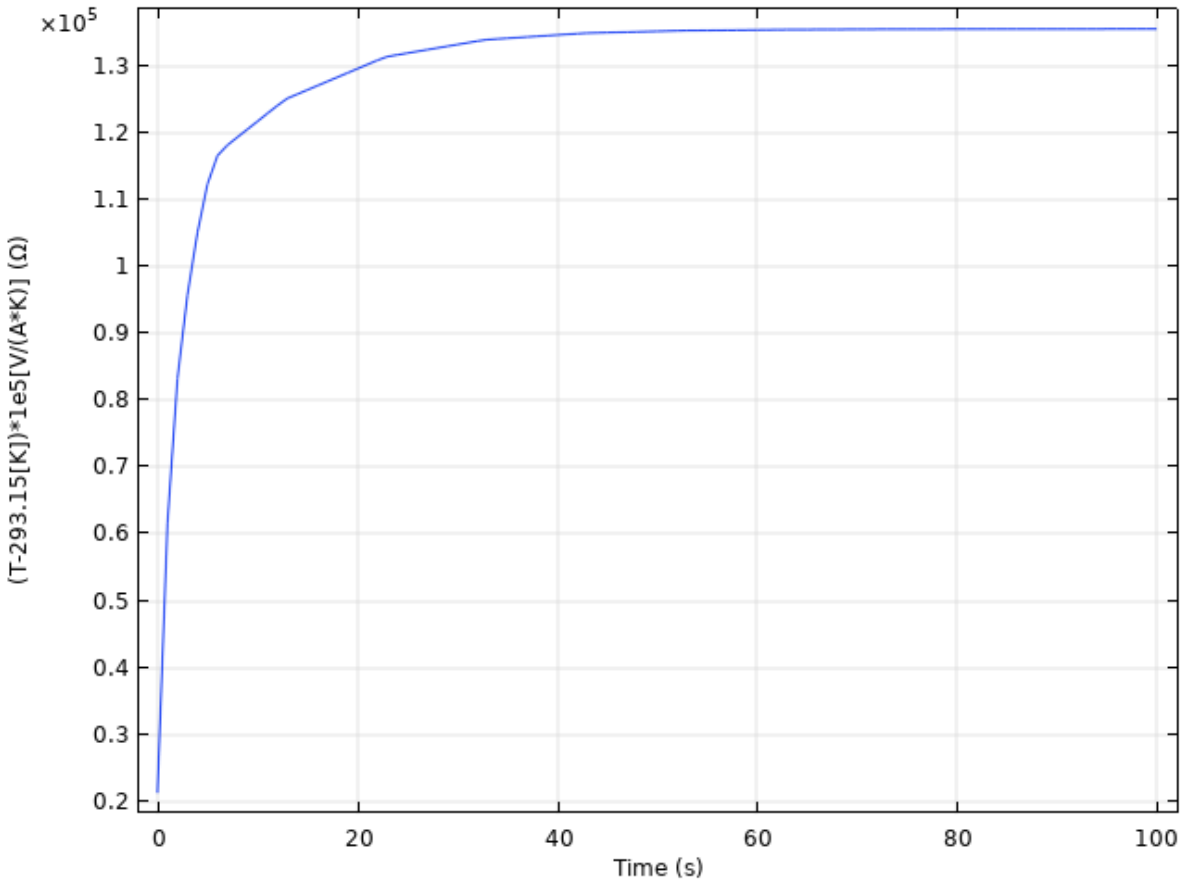

Figure 4.12: Average estimated ohmic evolution .

4.4.1 Discussion

According to the simulation the temperature increases in the microchannel of 1.5 kelvin (293.15 k to 294.63 k). This increase is due to the release of heat caused by the reaction that took place between the enzyme and the substrate within the microchannel. The temperature reaches its maximum at 100s.

General conclusion

This master's work consists of studying the functioning of a biosensor based on fixed enzymes in a microchannel in order to develop a MEMS biosensor application.

First, we started with a literature review of biosensors citing their types and principle of working of each type .

Next we defined the techniques of immobilization enzyme in General and specially Immobilization of Enzyme on Microchannel Surface .

We modeled an enzymatic biosensor and implemented this model on COMSOL Multiphysics digital simulation software based on the finite element method. Several simulation results have been shown and discussed.

We modeled an enzymatic biosensor and implemented this model on COMSOL Multiphysics digital simulation software based on the finite element method. Several simulation results have been shown and discussed.

In this work we have treated only one molecules (Cholesterol) . So this work should be continued in order to provide more precision as to the nature of the enzyme-substrate reactions. Specific case studies will have to be made as well as identification of immobilization processes in polymers (PDMS) will need to be identified precisely.

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